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# ASSOCIATIONS OF MICROORGANISMS IN RELATION TO SEEDLING INJURY ARISING FROM INFECTED SEED[1]

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## INTRODUCTION

Recent studies have emphasized the importance of the association of microorganisms in the soil in relation to foot and root rots of cereals. It has been shown that certain saprophytic fungi and bacteria inhibit the development of these diseases.

Henry (6) has demonstrated that seedling injury caused by *Helminthosporium sativum* P. K. and B. and *Fusarium graminearum* Schwabe is reduced by the antibiotic action of the soil microflora. Sanford and Broadfoot (9) found that the microflora of the soil had a deleterious effect on the development of root rot caused by *Ophiobolus graminis* Sacc. Garrett (4) concluded that the differences in soil microflora may account for the variations in prevalence of *Ophiobolus graminis*. Bisby, James, and Timonin (1) showed that *Trichoderma lignorum* (Tode) Harz. suppressed the virulence of *H. sativum* and *F. culmorum* (W. G. Sm.) Sacc. on wheat, and Greaney and Machacek (5) found that *Cephalothecium roseum* Corda also inhibited the pathogenicity of *H. sativum*.

Sanford and Broadfoot (9) and Brömmelhues (2) have shown that under certain conditions saprophytic or antagonistic organisms may actually increase the severity of infection caused by *Ophiobolus graminis*.

Numerous field experiments at St. Paul, Minnesota, proved that seedlings from seed artificially inoculated with, or planted in soil inoculated with, *Fusarium* spp. or *Helminthosporium* spp. seldom were injured. However, when naturally infected seed of wheat and barley was sown there was usually considerable foot and root rot. There is virtually no information on the effect of the microflora of the soil on the development of seedling blight or root rot arising from naturally infected seed. If the soil microflora prevents or appreciably inhibits seedling injury that usually arises from in-

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[2] The writer is indebted to Dr. Helen Hart for her assistance and criticism in preparing this paper.

fected seed, it would be of considerable economic importance. It would greatly minimize the importance of treating seed grain infected with foot- and root-rotting organisms. In Minnesota, wheat seed, and particularly barley seed, is often heavily infected with *Fusarium* spp. and *Helminthosporium* spp., the organisms commonly associated with seedling injury (3). Therefore, the effect of the association of microorganisms on the development of seedling blight and root rot resulting from sowing naturally infected barley seed was studied.

## METHODS

Most of the organisms used in these tests were isolated from diseased barley seed. Those designated as soil cultures were obtained from soil continuously cropped to barley for 15 years. The soil organisms were obtained by adding about 10 grams of soil to sterile oat hulls in Erlenmeyer flasks, resulting, of course, in very mixed cultures. The organisms, unless otherwise stated, were increased on ground oat hulls to which were added one-half per cent sucrose and usually a small piece of potato. In most cases only young, vigorous cultures were used for inoculation.

Four methods of inoculation were used: (1) dipping or soaking the seed in a suspension of spores and mycelial fragments or extracts of the organisms, (2) pouring the suspension over the seed in the soil, (3) adding the culture of the organisms to soil at, or previous to, the time of planting, and (4) dusting the seed with spores and mycelial fragments. Spore suspensions were strained through cheesecloth or a fine mesh screen to remove pieces of oat hull. Whenever a culture was added to the soil an excess was purposely avoided, and the inoculum was mixed thoroughly with the soil. In the greenhouse tests 2 kinds of soil were used: coarse sand and a mixture of 1 part black loam and 3 parts sand. The soil was autoclaved in shallow pans at about 12 pounds' pressure for from 5 to 7 hours.

Most of the diseased seed was obtained by spraying barley plants growing under muslin tents. Lot F was sprayed with many races of *Fusarium* spp.; lot M with numerous species of fungi other than *Fusarium* spp., mostly *Helminthosporium* spp.; and lot C was not sprayed and was virtually free from root-rotting fungi. Samples of diseased seed, lots A and H, were obtained from farmers.

In order to obviate environmental differences, pots or flats always were randomized and their position usually shifted at intervals of 2 or 3 days. Plots in the field also were randomized.

## EXPERIMENTAL RESULTS

*Tests with Trichoderma lignorum.*—In recent years the writer has made extensive tests on the effect of seed treatment, especially on barley infected

with various Fungi Imperfecti. In a test on varietal response to Ceresan a portion of the seed was dusted with spores and mycelial fragments of *Trichoderma lignorum*. It seemed probable that the lots responding to Ceresan treatment also might respond to the effect of the association with *T. lignorum*, as it is known that the benefit of Ceresan treatment is highly correlated with the severity of seed infection with Fusarium and Helminthosporium.

Germination tests were made on 2 seed lots, F and M, and plant vigor was noted on 3 lots, C, F, and M. A portion of each seed lot was treated with Ceresan at the rate of 2 oz. per bu., a second portion was dusted with spores and mycelial fragments of *Trichoderma lignorum*, an excessive amount of the fungus material being used, and a third portion remained untreated. Plating tests of seed on nutrient agar at the time of planting indicated that the *T. lignorum* and the pathogens were viable. The grain was sown in 4-foot rows, 100 seeds to the row. Each plot consisted of 3 rows, 1 with Ceresan-treated seed, 1 with *T. lignorum*-dusted seed, another as control. The varieties were replicated several times, and these are listed separately in table 1, while the 54 varieties (including the 5 mentioned varieties) were replicated but once and are considered as a composite group.

According to Weindling (10), the lethal principle of *Trichoderma lignorum* does not accumulate in any considerable quantity, and it is produced most abundantly when the fungus is about 2 days old; consequently, the application of conidia of *T. lignorum* to the seed at time of sowing should be ideal for antibiotic action. It is evident from the data given in table 1 that *T. lignorum* did not increase the average percentage of plants that emerged in the composite group, while Ceresan increased the percentage by 4 per cent for lot F and 10 per cent for lot M. These figures are statistically significant. Lot C did not respond to the Ceresan treatment. Of the five varieties listed in table 1, Peatland, with an average increase in stand of 22 per cent, responded most to Ceresan treatment.

Notes were taken on the relative vigor of plants in the seedling and early boot stages. There was close agreement between the two readings, indicating that the increased vigor persisted beyond seedling stage. Data on plant vigor were taken when plants were about 6 weeks old and indicate that Ceresan caused pronounced increase, while *Trichoderma lignorum* was ineffective (Table 1 and Fig. 1). The average increase in vigor of plants from Ceresan-treated seed was 12, 23, and 0 per cent for lots F, M, and C, respectively, of the 54 varieties.

To determine more accurately the relative vigor of the plants, the green weights of the 5 varieties were obtained. The plants were cut off at the ground line when about 6 weeks old and weighed immediately. Ceresan treatment increased the green weights of plants from seed lots F, M, and C

TABLE 1.—*The effect of treatment with Ceresan or inoculation with Trichoderma lignorum on germination and growth of barley from clean and blighted seed planted in the field*[a]

| Variety | Number of tests | | | Treatment and percentage of plants emerged | | | | | | | | | Treatment and percentage increase or decrease in vigor over checks | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ceresan | | | Control | | | Trichoderma | | | Ceresan | | | Trichoderma | | |
| | F[b] | M | C | F | M | Av. | F | M | Av. | F | M | Av. | F | M | C | F | M | C |
| Glabron | 10 | 10 | 3 | 41 | 63 | 52 | 33 | 54 | 44 | 39 | 53 | 46 | +21 | +7 | +2 | +7 | +5 | 0 |
| Lion | 8 | 8 | 5 | 14 | 46 | 30 | 15 | 31 | 23 | 13 | 36 | 25 | −7 | +34 | 0 | −3 | +6 | +7 |
| Manchuria | 10 | 10 | 5 | 47 | 62 | 55 | 44 | 47 | 46 | 41 | 44 | 43 | +10 | +22 | −1 | −4 | −2 | +2 |
| Minsturdi | 10 | 10 | 5 | 36 | 50 | 43 | 27 | 42 | 35 | 27 | 38 | 33 | +21 | +23 | −1 | +7 | −8 | +2 |
| Peatland | 10 | 10 | 5 | 56 | 90 | 73 | 45 | 56 | 51 | 47 | 64 | 56 | +21 | +25 | 0 | +7 | −4 | 0 |
| Composite of 54 varieties[c] | 2 | 2 | 1 | 32 | 49 | 41 | 28 | 39 | 34 | 28 | 37 | 33 | +12 | +23 | 0 | +.4 | 0 | +2 |

[a] Results are based on randomized rows each sown with 100 seeds, and the data on vigor of growth were taken on plants six weeks old.

[b] Seed lot F was obtained from plants sprayed with *Fusarium* spp. while growing under muslin tents; seed lot M from plants sprayed in a similar manner with numerous species of fungi other than Fusarium; and seed lot C was from plants grown in the open and not sprayed.

[c] This also includes the five varieties listed in the table.

FIG. 1. The effect of treatment with Ceresan and inoculation with *Trichoderma lignorum* on growth of barley from infected seed planted in the field.

by 29, 56, and 0.3 per cent, respectively; similarly, *T. lignorum* increased the green weights by 7, 3, and 1 per cent, respectively. While the figures for *T. lignorum* were not statistically significant, those for the Ceresan lots, with the exception of C, are highly so. The results obtained with Ceresan treatments agree with those of previous tests, namely, that the benefit from seed treatment of barley is approximately proportional to the degree of infection on or in the seed (3).

In view of the negative results with *Trichoderma lignorum* it seemed possible that the soil was infested with microorganisms that inhibited its growth or that Trichoderma and other organisms were already present in such abundance that the addition of more Trichoderma had no effect. Therefore, experiments were made in sterilized soil in the greenhouse.

In the first test, 2 seed lots of barley, F and H, infected primarily by *Fusarium* spp. and *Helminthosporium* spp., respectively, were used. In addition to the effect of *Trichoderma lignorum*, the effect of a mixed culture of fungi and bacteria obtained from soil was studied. Three different controls were used: (1) sterile oat hulls added to the soil, (2) seed treated with Ceresan, and (3) untreated seed. The sterile oat hulls and the 2 cultures were added to the soil at the time of planting. The results are based on randomized, quadruplicate, 7-inch pots each sown with 25 seeds. Counts were taken on the percentage of seedlings that emerged, the percentage distinctly injured (stunted or deformed), and the green weights when the plants were 1 month old. Henry (7) has shown that high temperatures are conducive

to antibiotic action of certain microorganisms. Consequently, tests were made at relatively high temperatures, which also are conducive to the development of foot and root rot caused by *Fusarium* spp. and *Helminthosporium* spp. In this test, the addition of *T. lignorum* to the soil or the treatment of seed with Ceresan did not materially affect the emergence. *Trichoderma lignorum* did not decrease the number of stunted or deformed plants, but Ceresan did reduce materially seedling injury. This becomes even more evident when one considers the data on green weight. Ceresan increased the green weight of lots F and H 31 and 23 per cent, respectively. In lot F, *T. lignorum* decreased the green weight by 11 per cent, and in lot H increased it 3 per cent. These differences probably are not significant.

The results in table 2 indicate that the addition of sterile oat hulls to the soil causes seedling injury and decreases the vigor of the plants. Sallans (8) found that wheat seedlings were severely injured if sterilized oat hulls were added to nonsterilized soil but not if added to sterilized soil. He attributed this injury not to the toxic substances in the medium but to the growth of the soil-inhabiting organisms upon the sterile material. In the present study it seems likely that the parasites on the seed spread to the oat hulls in sterilized soil, grew rapidly, and attacked the young seedlings.

TABLE 2. *The effect of various treatments on germination and growth of barley from blighted seed planted in sterilized soil*[a]

| Treatment of sterilized soil | Percentage of plants | | | | Green weight (in grams) | |
|---|---|---|---|---|---|---|
| | Emerged | | Stunted or deformed | | | |
| | F[b] | H | F | H | F | H |
| None—control | 61 | 76 | 21 | 14 | 5.4 | 9.8 |
| Sterile oat hulls added | 58 | 76 | 33 | 26 | 4.8 | 7.7 |
| *Trichoderma lignorum* added | 64 | 81 | 21 | 15 | 4.8 | 10.1 |
| Mass culture (from soil) added | 59 | 80 | 25 | 19 | 5.4 | 8.1 |
| None—seed treated with Ceresan | 63 | 76 | 8 | 7 | 7.1 | 12.1 |

[a] Results are based on randomized quadruplicate pots each sown with 25 seeds.

[b] F and H were infected chiefly with *Fusarium* spp. and *Helminthosporium* spp., respectively.

A similar experiment was made on sterilized and nonsterilized soil, using the same seed lots, F and H, and also another lot, A, which was badly discolored by fungi, chiefly *Alternaria* spp. These results are summarized in table 3. As in previous tests, the addition of *Trichoderma lignorum* cultures to sterilized and nonsterilized soil did not increase germination, seedling vigor, or green weight. However, the percentage of seedlings stunted or deformed in seed lots F and H were somewhat reduced by the addition of

TABLE 3.—*The effect of various treatments on germination and growth of barley from blighted seed planted in sterilized and non-sterilized soil*

| Treatment of soil and material added | Percentage of plants[a] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Emerged | | | | | | Stunted or deformed | | | | Green weight (in grams) | | | |
| | Nonsterilized | | | Sterilized | | | Nonsterilized | | Sterilized | | Nonsterilized | | Sterilized | |
| | A[b] | F | H | A | F | H | F | H | F | H | A | F + H (Av.) | A | F + H (Av.) |
| Control | 90 | 62 | 71 | 92 | 60 | 91 | 48 | 34 | 37 | 24 | 10.0 | 11.3 | 17.4 | 15.1 |
| Sterile oat hulls | 83 | 59 | 82 | 93 | 58 | 78 | 31 | 27 | 44 | 31 | 8.4 | 11.6 | 17.4 | 13.8 |
| *Trichoderma lignorum* | 90 | 52 | 89 | 91 | 66 | 85 | 40 | 16 | 30 | 15 | 9.9 | 11.2 | 15.1 | 13.4 |
| Mass culture (from soil) | 84 | 51 | 80 | 93 | 62 | 84 | 29 | 27 | 10 | 18 | 10.1 | 12.1 | 15.6 | 17.5 |
| Ceresan | 93 | 68 | 91 | 93 | 74 | 91 | 26 | 25 | 19 | 23 | 10.1 | 15.0 | 15.4 | 16.6 |

[a] Results are based on randomized quadruplicate pots each sown with 25 seeds.

[b] Seed lot A was discolored by a miscellaneous group of fungi, chiefly *Alternaria* spp., while seed lots F and H were infected chiefly with *Fusarium* spp. and *Helminthosporium* spp., respectively.

*T. lignorum* to both the sterilized and nonsterilized soil. Seed lot A developed virtually no seedling blight.

It is possible that the race of *Trichoderma lignorum* used was not particularly antibiotic to fungi on the seed, but the following tests proved, on the contrary, that it was distinctly antibiotic toward four races of *Helminthosporium sativum* isolated from seed. Clean seed of barley was inoculated by dipping it in a heavy spore suspension of *H. sativum* or one of *H. sativum* and *T. lignorum*. One portion was sown in sterile soil and another in sterile soil that had been inoculated 24 hours previously with a spore suspension of *T. lignorum*. Pots were randomized and sown in duplicate, each with 50 seeds. The results are given in table 4 and are in accord with those of other workers. There were better stands and fewer stunted or deformed seedlings when the seed was inoculated with a spore suspension of *H. sativum* and *T. lignorum* or when it was sown in soil to which *T. lignorum* was added. Plants of these lots also were more vigorous than those of the control that had been inoculated with *H. sativum* alone. Trichoderma was distinctly antibiotic to *H. sativum*.

The antibiotic effect of *Trichoderma lignorum* on several species of Helminthosporium was tested in other experiments, and the results were similar. There were indications that the less virulent the race or species of Helminthosporium the more pronounced was the effect of *T. lignorum*.

Races of *Trichoderma lignorum* may differ in antibiotic effect, however, for a race given to the writer by I. Tervet[3] was less antibiotic toward *H. sativum* than the one used in this work. Differences in antibiotic action among races of *T. lignorum* was noted by Weindling (10).

*Tests with other organisms.*—A number of investigators have shown that several organisms besides *Trichoderma lignorum* may inhibit the development of root rot. Moreover, there is sometimes a high degree of specificity in antibiotic action among fungi. Therefore, experiments were made on the antibiotic effect of several microorganisms isolated either from the seed or soil. These tests were made in a manner similar to those described for *T. lignorum*.

As with *T. lignorum*, the most extensive tests were made in the field. In one test 3 seed lots from 2 varieties of barley were used, and 100 seeds of each lot were sown in randomized quadruplicate rows. Severely infected seed lots were planted so that the effect of the association of microorganisms could be more easily ascertained. At the time of planting, the seeds were immersed in heavy suspensions of spores and mycelial fragments of the respective organisms. Notes were taken on percentage stand and vigor of plants, and at the end of the experiment the green weights were determined. The data in table 5 indicate that none of the 12 organisms used influenced

[3] Commonwealth Fellow 1933–1934, University Farm, St. Paul, Minnesota.

TABLE 4.—*The effect of Trichoderma lignorum on germination and growth of barley from clean seed inoculated with four races of Helminthosporium sativum planted in sterilized soil*

| Races of *H. sativum* | Percentages of plants[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Emerged | | | Stunted | | | Contorted leaves | | |
| | H[b] | H + T | H + S I | H | H + T | H + S I | H | H + T | H + S I |
| 21 | 84 | 94 | 94 | 46 | 12 | 6 | 52 | 32 | 15 |
| 22 | 88 | 94 | 98 | 33 | 8 | 6 | 57 | 27 | 14 |
| 23 | 86 | 88 | 96 | 25 | 17 | 8 | 78 | 31 | 21 |
| 24 | 88 | 98 | 94 | 10 | 4 | 3 | 17 | 15 | 10 |

[a] Results are based on randomized duplicate pots each sown with 50 seeds.

[b] H = Seed inoculated with a spore suspension of *Helminthosporium sativum*.

H + T = Seed inoculated with a spore suspension of *Helminthosporium sativum* in combination with *Trichoderma lignorum*.

H + S I = Seed inoculated with a spore suspension of *Helminthosporium sativum* and planted in soil inoculated with *Trichoderma lignorum*.

the stand or green weight. On several occasions notes on the relative vigor of the plants were taken, but there were never any indications that the organisms increased or decreased the vigor of the plants; in this respect, however, the effect of the Ceresan treatment was very striking.

A similar test also was made with 5 organisms, using 2 lots of diseased barley from 5 varieties: Glabron, Manchuria, Minsturdi, Peatland, and Wisconsin No. 38. Each lot was sown in duplicate randomized rows, 100 seeds to a row. The data are summarized in table 6. Although considerable seedling injury occurred, there was no beneficial effect from the addition of 5 microorganisms. It is noteworthy that the amount of seedling blight arising from naturally infected seed was not increased by the addition of *Fusarium culmorum* to the seed at time of planting. Apparently the antibiotic organisms in the soil inhibited *F. culmorum* when seed was artificially inoculated but were ineffective when seed was naturally infected.

In still another test large quantities of all the organisms mentioned in table 5, with the exception of *Fusarium culmorum*, were applied to the soil. Three diseased lots of barley seed, comprising 2 varieties of barley, were sown in open furrows to which was added about 500 cc. of oat-hull cultures. Each seed lot was replicated 4 times. Considerable seedling blight developed. The addition of the large quantities of the mixed cultures at seed level did not prevent seedling injury, while again Ceresan was decidedly effective.

The inhibiting effect of several microorganisms on seedling blight arising from infected seed also was studied in the greenhouse. Minsturdi seed infected naturally with *Fusarium* spp. and *Helminthosporium* spp. and also

TABLE 5.—*The effect on germination and growth of barley from blighted seed inoculated with twelve organisms and planted in the field*[a]

| Inoculum | Stand in percentage | | | | Green weight (in grams) | | | |
|---|---|---|---|---|---|---|---|---|
| | Minsturdi | | Minn. No. 462 | Average of 3 lots | Minsturdi | | Minn. No. 462 | Average of 3 lots |
| | F[b] | M | F | | F | M | F | |
| Control—A | 31 | 39 | 35 | 35 | 145 | 214 | 203 | 187 |
| Bacterium | 22 | 41 | 42 | 35 | 114 | 195 | 250 | 186 |
| *Actinomyces scabies* | 29 | 46 | 38 | 38 | 115 | 214 | 232 | 187 |
| *Aspergillus clavatus* | 32 | 45 | 42 | 40 | 154 | 239 | 233 | 209 |
| *Aspergillus giganteus* | 30 | 40 | 35 | 35 | 147 | 210 | 194 | 184 |
| *Aspergillus terreus* | 30 | 43 | 45 | 39 | 136 | 256 | 255 | 216 |
| *Cephalothecium roseum* | 30 | 47 | 32 | 36 | 137 | 225 | 253 | 205 |
| *Chaetomium spirochaete* | 31 | 39 | 38 | 36 | 211 | 215 | 233 | 220 |
| *Cunninghamella* sp. | 31 | 40 | 30 | 34 | 132 | 275 | 188 | 198 |
| *Fusarium culmorum* | 37 | 43 | 33 | 38 | 185 | 223 | 237 | 215 |
| *Penicillium expansum* | 33 | 44 | 36 | 38 | 116 | 226 | 235 | 192 |
| *Penicillium martensii* | 30 | 41 | 36 | 36 | 168 | 202 | 199 | 190 |
| *Trichoderma lignorum* | 33 | 48 | 39 | 40 | 163 | 241 | 219 | 208 |
| Mixture of above organisms[c] | 34 | 52 | 41 | 42 | 145 | 281 | 233 | 220 |
| Control—B | 34 | 43 | 41 | 39 | 161 | 248 | 194 | 201 |

[a] Results are based on randomized quadruplicate rows each sown with 100 seeds.

[b] Seed lot F was obtained from plants sprayed with *Fusarium* spp. while growing under muslin tents, and seed lot M was from plants sprayed in a similar manner with numerous species of fungi other than *Fusarium*.

[c] Except *Fusarium culmorum*.

TABLE 6.—*The effect on germination and growth of barley*[a] *from blighted seed inoculated with five organisms and planted in the field*

| Inoculum | Percentage of plants emerged | | Green weight | | | |
|---|---|---|---|---|---|---|
| | | | In grams | | Percentage increase or decrease over control | |
| | F | M | F | M | F | M |
| Bacterium | 31 | 34 | 231 | 209 | −15 | −13 |
| *Actinomyces scabies* | 32 | 37 | 241 | 241 | −11 | + 1 |
| *Cephalothecium roseum* | 31 | 36 | 258 | 227 | − 5 | − 5 |
| *Fusarium culmorum* | 31 | 33 | 248 | 231 | − 9 | − 3 |
| *Penicillium expansum* | 34 | 35 | 276 | 246 | + 1 | + 3 |
| Control | 35 | 33 | 272 | 239 | – | – |

[a] Results are based on the averages of 5 varieties (Glabron, Manchuria, Wis., No. 38, Minsturdi, and Peatland), each grown in randomized duplicate plots.

Minn. No. 462 infected with *Fusarium* spp. were sown in flats containing sterilized sand. The cultures of 12 organisms were added separately to sand in flats 2 days before planting. The seed was sown in randomized duplicate rows, 50 seeds in each row. The results are summarized in table 7. As in the field tests, the addition of microorganisms to the soil was ineffective in reducing seedling injury.

TABLE 7.—*The effect on germination and growth of barley from blighted seed planted on sterilized soil inoculated with twelve organisms forty-eight hours before planting*

| Inoculum | Percentages of plants [a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Emerged | | | | Stunted or deformed | | | |
| | Minsturdi | | Minn. No. 462 | Average of 3 lots | Minsturdi | | Minn. No. 462 | Average of 3 lots |
| | F | M | F | | F | M | F | |
| Bacterium | 54 | 93 | 77 | 75 | 18 | 7 | 14 | 13 |
| *Actinomyces scabies* | 52 | 85 | 77 | 71 | 20 | 8 | 14 | 14 |
| *Aspergillus clavatus* | 57 | 84 | 81 | 74 | 10 | 12 | 12 | 11 |
| *Aspergillus giganteus* | 56 | 89 | 80 | 75 | 16 | 12 | 19 | 16 |
| *Aspergillus nidulons* | 51 | 86 | 78 | 72 | 28 | 9 | 13 | 17 |
| *Cephalothecium roseum* | 60 | 83 | 72 | 72 | 25 | 12 | 19 | 19 |
| *Chaetomium spirochaete* | 64 | 87 | 80 | 77 | 12 | 12 | 12 | 12 |
| *Cunninghamella* sp. | 63 | 87 | 74 | 75 | 14 | 12 | 18 | 15 |
| *Penicillium elongatum* | 63 | 93 | 73 | 76 | 17 | 11 | 24 | 17 |
| *Penicillium expansum* | 61 | 88 | ..... | ..... | 19 | 13 | ..... | ..... |
| *Penicillium martensii* | ..... | 91 | 68 | ..... | ..... | 12 | 19 | ..... |
| *Trichoderma lignorum* | 50 | 89 | 80 | 73 | 16 | 11 | 18 | 15 |
| Sterile oat hulls | 49 | 87 | 79 | 72 | 20 | 11 | 13 | 15 |
| Control | 61 | 89 | 81 | 77 | 18 | 6 | 20 | 15 |

[a] Results are based on randomized duplicate rows each sown with 50 seeds.

Sanford and Broadfoot (9) found that filtrates from certain organisms gave greater protection than the living cultures. In order to test the efficacy of extracts of microorganisms, diseased grain of Peatland barley was immersed for 4 hours in a water extract of 5 organisms. The extract was made by chopping up the culture and soaking it in sterilized water for 24 hours. This was then strained through cheesecloth; hence the extract contained also viable spores and fragments of mycelium, or bacteria. The seed was sown in randomized quadruplicate rows, 50 seeds in each. The data are presented in table 8. *Actinomyces scabies* (Thaxt.) Güss. and *Trichoderma lignorum* apparently decreased the number of diseased plants in lot F infected with Fusarium but not in lot M infected primarily with Helminthosporium. In fact, Trichoderma appears to be injurious to this lot, as the percentage of

TABLE 8.—*The effect on germination and growth of Peatland barley from blighted seed soaked four hours in an extract of five different organisms and planted in sterilized soil*

| Inoculum | Percentages[a] | | | | | |
|---|---|---|---|---|---|---|
| | Stand | | Stunted | | Disease-free | |
| | F[b] | M | F | M | F | M |
| Bacterium | 56 | 95 | 29 | 33 | 28 | 9 |
| *Actinomyces scabies* | 52 | 95 | 21 | 27 | 45 | 11 |
| *Aspergillus nidulans* | 56 | 94 | 26 | 23 | 35 | 16 |
| *Cephalothecium roseum* | 56 | 94 | 27 | 31 | 36 | 10 |
| *Trichoderma lignorum* | 55 | 86 | 20 | 27 | 45 | 19 |
| Control | 60 | 96 | 26 | 24 | 34 | 16 |

[a] Results are based on randomized quadruplicate rows, each sown with 50 seeds.

[b] Seed lot F was obtained from plants sprayed with *Fusarium* spp. while growing under muslin tents, and seed lot M was from plants sprayed in a similar manner with numerous species of fungi other than Fusarium.

plants that emerged was decreased 10 per cent. Perhaps if higher concentration of the extracts had been tried or if the period of immersion had been increased, the results might have been different.

The antibiotic action of several organisms to *Helminthosporium sativum* in sterilized soil was proved by inoculating virtually disease-free barley seed with a mixture of a spore suspension of *H. sativum* and several other organisms. The results are given in table 9 and are based on randomized quadruplicate pots each sown with 25 seeds. All of these organisms increased the number of plants that emerged, although some more than others.

TABLE 9.—*The effect on germination and growth of barley from clean seed inoculated with Helminthosporium sativum in combination with six other organisms and planted in sterilized soil*

| Inoculum added to *H. sativum* | Percentage of plants[a] | |
|---|---|---|
| | Stand | Stunted or deformed |
| None—*H. sativum* only | 80 | 36 |
| *Bacterium* | 83 | 17 |
| *Basisporium gallarum* | 89 | 25 |
| *Cephalothecium roseum* | 88 | 31 |
| *Chaetomium spirochaete* | 88 | 14 |
| *Penicillium* sp. | 91 | 31 |
| *Trichoderma lignorum* | 81 | 25 |
| Control—uninoculated | 97 | 3 |

[a] Results are based on randomized quadruplicate pots each sown with 25 seeds.

All of them reduced the percentage of stunted seedlings; *Chaetomium spirochaete* Palliser and the bacterium were especially effective.

Apparently, there are a number of organisms that have antibiotic effect on *Helminthosporium sativum* on the seed or in the soil. If, however, *H. sativum* and other fungi are within the seed coat or have invaded the tissues of the seedling, the antibiotic organisms have little or no effect.

## DISCUSSION

The results of these experiments indicate that the microflora of the soil at University Farm, St. Paul, did not prevent or appreciably decrease seedling blight or root rot arising from infected barley seed. Preliminary tests with infected wheat and oats gave similar results. The behavior of infected seed lots was quite similar whether sown in the field or greenhouse, in sterilized or nonsterilized soil. Under all conditions studied, diseased seed always germinated poorly or weakly and developed considerable seedling blight and foot and root rot, while clean seed usually developed healthy plants. The fact that treatment of barley seed is generally beneficial in proportion to the extent of seed infection also is an indication that the primary source of seedling injury often is infected seed. This may not be true under certain cultural practices, as sowing barley on disced cornstalk land. In certain years it is not uncommon to have barley grain with 10 to 25 per cent or more infection by Fusarium, Helminthosporium, and other root-rotting organisms. Naturally, the sowing of seed infected with virulent parasites is not uncommon. It is quite possible, however, when barley seed is only lightly infected, that the soil microflora may suppress and even prevent seedling injury. This certainly is true when the pathogen is applied directly to the seed or to the soil. Obviously, the soil microflora plays an important rôle in suppressing or eliminating soil-borne parasites, and the importance of proper rotation should not be minimized.

The results obtained are of considerable practical significance. They help to explain, at least in part, why considerable Fusarium and Helminthosporium seedling injury may sometimes develop when good rotation is practiced and even on summer-fallowed land. They emphasize the necessity of treating seed, so as to eliminate insofar as possible the pathogen on or in the seed. They also point out the importance of sowing disease-free barley seed when studying the effect of various cropping systems on the development of root rot. It is important to remember that, although seed treatment will eliminate much of the primary infection, it will not prevent secondary infection. Eventually, all barley and wheat plants, at least under Minnesota conditions, become more or less attacked by soil-borne parasites, but the greatest injury from Fusarium and Helminthosporium usually results from early seedling infection. Although most of the seed treatment tests were made

with ordinary Ceresan, additional tests with New Improved Ceresan at the rate of one-half ounce per bushel have given equally good results.

SUMMARY

The results indicate that the soil microflora does not have a marked effect on seedling blight arising from infected barley seed. There were no differences in germination or amount of seedling injury when barley seed naturally infected with Helminthosporium or Fusarium was planted in sterilized and nonsterilized soil.

Antibiosis is perhaps of little importance in suppressing seedling injury from diseased barley seed. The addition of *Trichoderma lignorum* and several other fungi and bacteria to naturally infected barley seed or to sterilized or nonsterilized soil did not inhibit or delay the parasitic action of seed-borne parasites. Soaking infected seed in an extract of these organisms also gave negative results.

The addition of *Trichoderma lignorum* and certain other fungi and bacteria to seed or sterilized soil inoculated with *Helminthosporium sativum*, however, increased the stand, decreased the number of deformed and stunted plants, and suppressed seedling injury.

Naturally infected barley seed is one of the primary sources of seedling injury in Minnesota and should not be sown unless properly treated.

Treating naturally infected seed with Ceresan improved the stand, decreased seedling injury, and materially increased plant vigor. Preliminary tests with New Improved Ceresan also gave good results.

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LITERATURE CITED

1. BISBY, G. R., N. JAMES, AND M. TIMONIN. Fungi isolated from Manitoba soil by the plate method. Canadian Jour. Res. 8: 253–275. 1933.
2. BRÖMMELHUES, M. Die wechselseitige Beeinflüssung von Pilzen und die Bedeutung Pilzkonkurrenz für das Ausmass der Schädigung an Weizen durch *Ophiobolus graminis* Sacc. Zentbl. Bakt. [etc.], Abt. II, 92: 81–116. 1934.
3. CHRISTENSEN, J. J., AND E. C. STAKMAN. Relation of Fusarium and Helminthosporium in barley seed to seedling blight and yield. Phytopath. 25: 309–327. 1935.
4. GARRETT, S. D. Factors affecting the severity of take-all. 1–3. Jour. Agr. South Australia 37: 664–674; 799–805; 976–983. 1934.
5. GREANEY, F. J., AND J. E. MACHACEK. Studies on the control of root-rot diseases of cereals caused by *Fusarium culmorum* (W. G. Sm.) Sacc. and *Helminthosporium sativum* P., K., and B. II. Pathogenicity of *Helminthosporium sativum* as influenced by *Cephalothecium roseum* Corda in greenhouse pot tests. Sci. Agr. 15: 377–386. 1935.
6. HENRY, A. W. The natural microflora of the soil in relation to the foot-rot problem of wheat. Canadian Jour. Res. 4: 69–77. 1931.

7. ————————. Influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis* Sacc. Canadian Jour. Res. 7: 198–203. 1932.

8. SALLANS, B. J. Methods of inoculation of wheat with *Helminthosporium sativum* P., K., and B. Sci. Agr. 13: 515–527. 1933.

9. SANFORD, G. B., AND W. C. BROADFOOT. Studies of the effects of other soil-inhabiting micro-organisms on the virulence of *Ophiobolus graminis* Sacc. Sci. Agr. 11: 512–528. 1931.

10. WEINDLING, R. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopath. 24: 1153–1179. 1934.

# COPPER DEFICIENCY IN SUGAR BEETS

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## INTRODUCTION

There are various practical examples of surplus yields obtained by the application of copper combinations. In part of the instances certain symptoms of disease even disappeared, as in the case of the so-called "reclamation disease" in Holland. This disease occurs in the Netherlands chiefly in the provinces Drente, Overijssel, Utrecht, Gelderland, Noord-Brabant and Limburg, and especially on heath land, which is very wet in the winter, or was so before the drainage, and dries up in the summer. On sandy soils, in cultivation for a few decades only, the disease appears to occur more frequently than on those longer in cultivation. It occurs also on peaty soils; particularly, on those not much used for peat cutting and of which the peat bog has more the character of lowland (moor) fen than of upland fen.

In Germany the disease is called "Heidemoorkrankheit" or "Urbarmachungskrankheit" (32, 33) or "Weisseuche" (4); in England it is indicated as "Reclamation disease" or "Heath bog disease," also "Yellow tip" (17).

This disease was described for the first time by Elema (5) as occurring in Holland in oats on reclaimed heath land. Barley also appeared to be very sensitive.

Meijer and Hudig (27) state as regards oats that, initially, the plants grow normally. However, as soon as they have formed a few leaves, yellow grayish spots suddenly turn up in the field, especially during warm, dry weather. The following day the leaves appear to have yellow whitish arid points in the ill spots. One or two days thereafter these points have become larger and are, especially when viewed from a distance, almost white. These symptoms are not always the same; sometimes the arid points are a bright white or are a little more yellow; there are cases in which the symptoms only appear at a later stage of growth. It occurs that the plants are more yellow or greener or that the leaves are striped yellow green and yellow. The plants recover a little during rainfall. Sometimes they die completely and in other instances they still form panicles containing empty or very light grains; the chaff is often white. The culms form new

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a dirty green and becomes finally brownish. The upper internodes of the culms are much too short but their leaves are normal. In cases of milder affection the plants grow normally until they shoot—sometimes even particularly well—but then they do not make further headway. The upper internodes of the culm do not develop. The ripening takes place in the same way as in the more serious cases. The grains are very light or empty. The stubble sends up secondary shoots. The disease could be controlled by 80,000 kg. of compost per hectare (27) or by copper sulphate (13, 14, 15).

Meijer and Hudig (27) intimated that the cause might be found in a kind of black moor or black heath bog, locally called "pik" or "gliede."

Smith (41) attributed the disease to an organic material with slightly acid qualities, which he termed "gliedine".

Only the investigations of Brandenburg (2) showed that the disease is simply the result of copper deficiency. In the absence of copper, symptoms were obtained in water cultures with oats that were exactly the same as those of the "reclamation disease" occurring in practice.

Sjollema (40) along other channels came to the same conclusion. In regions where the "reclamation disease" occurred, cattle were often found to suffer from the "licking disease", which is accompanied by anaemia. The symptoms are: considerable backwardness in development, marked emaciation, dryness of the hair, inclination to lick, to bite wood, etc., lack of appetite, consumption of extraordinary food, refusal of grass and, therefore, failure to graze normally, a muzzy, dreamy expression of the eyes. A cure can be quickly effected by means of copper sulphate. This implies that cattle on farms where the reclamation disease is prevalent absorb too little copper. Analyses of compost showed a copper content of 190–400 mg. per kilo, *i.e.*, when applying 80,000 kg. compost there is an amendment of 15.2–32 kg. of copper, which quantity corresponds to 61–130 kg. copper sulphate.

Meijer and Hudig (27) state that oats are most sensitive to this disease, followed by rye and black President oats; while potatoes, lupines, and serradella appear to be rather insensitive. Grassland loses the good grasses and retains chiefly *Holcus lanatus* and white clover. On very "sick" soil, potatoes and grass will not grow well, either.

On basis of field experiments of several years with various crops Meijer (28) arrives at the following conclusions:

1) The quantity of copper sulphate to be employed has relation with the humus content of the soil. In case of humus-rich soils it is safe to apply 100 kg. per ha (sulphate of copper testing 25 per cent Cu). Already in case of 7 per cent humus this may be necessary.

2) 50 kg. copper sulphate per ha is not always conclusive, but quantities of more than 100 kg. do not appear to be necessary.
3) Excess of copper sulphate may sometimes do harm; 50 kg. per ha may already be too much for sandy soils without humus.
4) Liming aggravates the reclamation disease.
5) An application of copper sulphate, efficient in relation to the humus content, (50 to 100 kg. per ha) is probably sufficient for at least 7 years.

In an experiment carried out at Drouwerveen in 1936 on a soil containing about 17 per cent humus and affected by the reclamation disease, a quantity of 50 to 150 kg. copper sulphate appeared to increase the crop of beets by about 44 per cent and the sugar yield by 60 per cent. 50 kg. per ha was adequate. The plants in the untreated fields seemed to have during the first 1½ months after coming up a lighter color than those dressed with copper sulphate. A month later the color of the leaves was normal and again 2 to 3 months later the foliage had a slightly greener color and was moreover shorter and steeper than that of the plants with copper sulphate. The growth of the beets of the untreated plots remained from the outset behind those of the treated fields. This trial field received shortly before the application of the seeds a quantity of farmyard manure of 25 tons per ha. It is not impossible that copper was added simultaneously with the farmyard manure and that consequently symptoms of disease in the plants without copper sulphate did not occur distinctly.

In the summer of 1935 symptoms of copper deficiency were studied in water cultures with sugar beets (38, 39).

## MATERIALS AND METHOD

Chemicals "Kahlbaum pro Analyse" previously twice recrystallized in double-distilled water, were employed for the water cultures. The water was distilled from stills of pyrex glass and also received in stills of pyrex glass. Also double distilled water was used for the solutions, whilst pyrex tumblers of 1 liter with paraffined plaster covers served as pots. In each pot one beet plant was grown.

The nutrient solution contained per liter: 1.00 g. nitrate of potash, 0.50 g. sulphate of magnesia, 0.50 g. sulphate of calcium, 0.70 g. phosphate of calcium (tertiary), and furthermore; 60 mg. ferro ammonium sulphate, 5 mg. sulphate of manganese, 0.7 mg. boric acid, 0.5 mg. sulphate of zinc.

The tertiary phosphate of calcium was obtained by conversion of phosphate of sodium and chloride of calcium, which also were previously twice of sodium and chloride of calcium, which also were previously twice crystallized. Ten pots received 0.5 mg. $CuSO_4$ 5aq., while 10 control pots received no copper. To make sure that the solutions to which no copper was applied were indeed free of copper, an oat plant (variety "Zege")

was grown beside each beet plant, as it is known that oats are very sensitive to copper deficiency and show very characteristic symptoms.

The seed of the beets (variety Hilleshög) was germinated before in good leaf-mould and transferred to the pots on June 26 after the formation of the first 2 heart leaves. Plants of equal size were selected and the roots carefully cleaned with double-distilled water.

The oat grains were kept for a week on filter paper in dishes and were kept moist by copper-free water. Three days after the transfer of the young beet plants they were placed in the solutions. The solutions were renewed every 4 weeks and regularly supplemented.

## RESULTS

With the aid of the oat plants, it could be shown that the solutions, to which no copper was applied, were indeed free of copper, for, as noted by Brandenburg (3), the first very typical symptoms of the disease appear in oats after the lapse of 4 weeks.

The beets without copper reacted already after 19 days very distinctly with a light chlorosis which increased gradually in intensity. This chlorosis began in most cases at the top of the leaves and spread fairly regularly over the whole leaf surface resulting in a marbled appearance. The veins contrasted as green against the light yellow greenish interstitial leaf tissue. The symptoms were clearest in the oldest leaves and those at half the height. In some cases the lower leaves were free of chlorosis, while the taller leaves were clearly marbled (Fig. 1, A). No chlorosis developed in the heart leaves. The green parts of the leaves showed sometimes a blue-greenish tint; in other cases the color was normal, *viz.*, dark green.

In the chlorotic parts of the older leaves the leaf tissue died after 2 months and became a gray brownish to gray or white. The chlorotic leaves were mostly a little thinner than the leaves of the healthy control plants and were sometimes a little arched marginally (Fig. 2, A). The leaves of the diseased plants were, during the first 6 weeks, in general, somewhat larger than the corresponding leaves of the control plants. Furthermore, the habit was on the whole rather normal. They produced only very little or no starch, which was demonstrated by treating leaves collected at 5 p.m. on July 26 (one month after planting) a few diseased and healthy leaves according to Sachs' iodine experiment, in which case the starch of the leaves becomes colored by the iodine, present in the solution of potassium iodine following removal of the chlorophyll by boiling with concentrated alcohol. The greatest accumulation of starch was observable in the veins of the diseased leaves, whilst the rest of the leaf tissue became slightly brown or remained unchanged as to color in contrast with the blue black discoloration of the leaf tissue of the healthy plants (Fig. 2, B). After a few weeks this

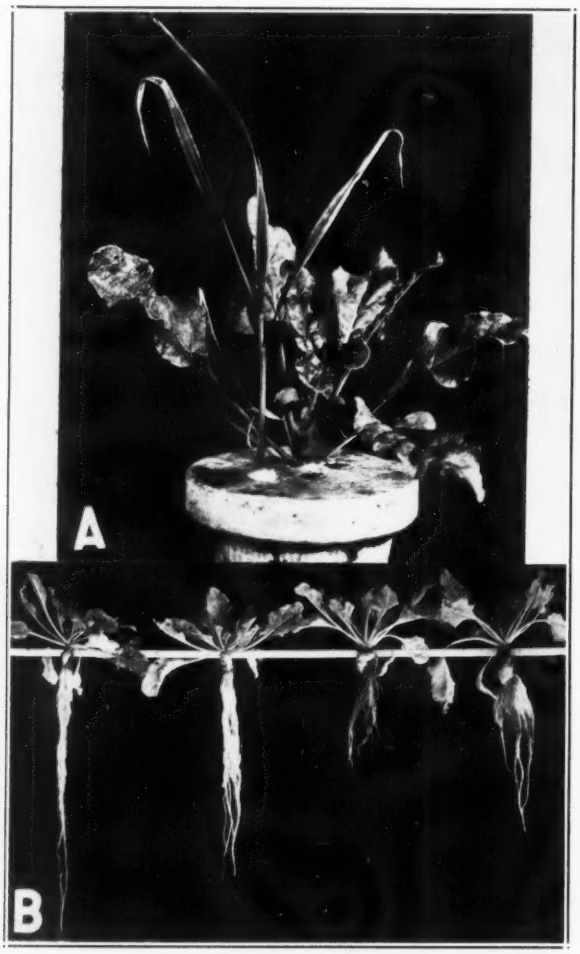


FIG. 1. A. Symptoms of copper deficiency in oats (variety "Zege") and in sugar beet (variety Hilleshög). The oats plant shows the white points in the leaves which are characteristic for the reclamation disease. The lower and the heart leaves of the sugar beet do not show chlorosis, but the others show marbling. B. The two beet plants on the left did not receive copper; those on the right received 0.5 mg. copper sulphate per liter. A distinct difference is noticeable between the root systems of the two series. Where no copper was applied the tap root remained very small, and the side roots became long and thin.

test for starch was repeated, with the same result. The heart leaves produced relatively the highest amount of starch in the diseased plants, though also distinctly less than in the sound healthy ones.

It may be concluded from this that copper is indispensable for beets and plays an essential role in the function of the chlorophyll, as is probably the

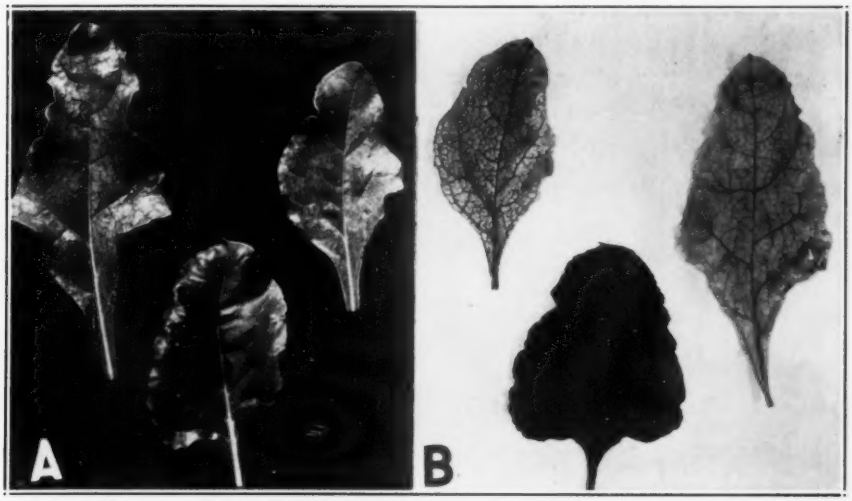


FIG. 2. A. Both upper leaves originated on two beet plants to which no copper was applied. They show the finely shaded chlorosis and have locally arched edges. The other leaves originated on a plant dressed with 0.5 mg. copper sulphate per liter. B. The same leaves after treatment according to Sachs' iodine experiment at 5 p. m. In the two upper leaves only the leaf veins became dark colored but the other leaf became blue-black over its whole surface.

case with the haemoglobin in bodies of animals (7, 8, 9, 10, 11, 18, 19, 20, 21, 22, 26, 29, 41).

In the absence of copper the lateral roots were generally longer and thinner than those of the control plants and pure white (Fig. 1, B). The tap root remained much retarded in development, compared with the tap roots of the sound plants, though the latter disposed, during part of the vegetation period, of relatively less food than the corresponding diseased plants, because the healthy oats growing beside the beets, probably had absorbed more food from the solution than the corresponding plants that received no copper.

To obtain a better comparative picture, the oat plants were removed on August 12. The diseased plants then had an average height of about 25 cm. and the healthy ones of about 45 cm. The beets were harvested on September 11 and the yields are presented in table 1. The great significance of the element copper for the beet appears clearly from the figures. In the first place the large difference in weight of roots is striking. The weight of the roots of the diseased plants is, on an average, $10.522 \pm 0.47$ grams and of the sound ones $30.597 \pm 1.4$ grams. The difference amounts to $20.075 \pm 1.48$ grams and is, therefore, reliable.

TABLE 1.—*Effect of copper on sugar beets in 1935. Duration of experiment, 71 days*

| | No. pot | Weight of roots | Average weight of roots | Weight of foliage | Average weight of foliage | Average sugar content | Average weight of sugar | Dry matter content roots | Ash content roots |
|---|---|---|---|---|---|---|---|---|---|
| | | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* | *grams* |
| No copper | 1 | 10.880 | | 15.780 | | | | | |
| | 2 | 11.650 | | 32.050 | | | | | |
| | 3 | 12.175 | | 29.825 | | | | | |
| | 4 | 13.100 | | 30.510 | | | | | |
| | 5 | 10.760 | | 25.175 | | | | | |
| | 6 | 9.605 | 10.522 | 19.590 | 24.411 | 15.2 | *1.60* | 24.73 | 1.050 |
| | 7 | 9.885 | ± 0.47 | 24.600 | ± 1.69 | | | | |
| | 8 | 9.910 | | 25.825 | | | | | |
| | 9 | 8.250 | | 20.250 | | | | | |
| | 10 | 9.005 | | 20.505 | | | | | |
| 0.5 mg. $CuSO_4$ 5 aq. p. L. | 11 | 27.800 | | 25.720 | | | | | |
| | 12 | 26.010 | | 24.020 | | | | | |
| | 13 | 24.960 | | 24.970 | | | | | |
| | 14 | 33.750 | | 26.720 | | | | | |
| | 15 | 37.275 | | 24.600 | | | | | |
| | 16 | 35.525 | 30.597 | 28.005 | 25.458 | 16.85 | *5.16* | 24.70 | 0.924 |
| | 17 | 35.725 | ± 1.47 | 25.620 | ± 0.419 | | | | |
| | 18 | 30.635 | | 24.870 | | | | | |
| | 19 | 25.525 | | 23.625 | | | | | |
| | 20 | 28.760 | | 26.430 | | | | | |

The relation of weight of foliage to weight of roots is, for the diseased plants, 2.32 and for the sound ones 0.83; therefore, in the first case, it is considerably larger than in the second one at this stage of growth.

The average sugar content of the diseased plants is 15.2 per cent and of the sound ones 16.85 per cent, so that the weights of sugar per beet are 1.60 and 5.16 grams, respectively. The plants that received copper, therefore, produced, after 71 days, an average of 3.2 times the quantity of sugar produced by the plants to which no copper was applied. The average weight of foliage of the 2 series does not differ much. But the difference would, no doubt, have been larger—also the difference in the sugar yield—had the experimental period been prolonged, because a few weeks before the harvest no noticeable growth could any longer be ascertained in the diseased plants. This was also the reason why the trial was terminated on September 11. The dry-matter and ash contents differ but little in the two series.

DISCUSSION

In the periodical "Mededeelingen" of the "Instituut voor Suikerbietenteelt" (Bergen of Zoom, Holland) (37), issue of January, 1936, the writer dealt with the principal diseases that, in Europe, cause yellow discoloration or symptoms of chlorosis in beets, *viz.:* Nitrogen deficiency; manganese deficiency; black wood-vessel disease, caused by Pythium sp.; verticilliosis or fusariosis; mosaic disease; and yellowing disease, to which should now possibly be added copper deficiency.

When comparing the symptoms of copper deficiency in beets with those of the Pythium-disease, described by Brandenburg (2), and called black wood-vessel disease by Quanjer (32), there is similarity insofar as in both cases a light yellow greenish discoloration is found between the veins of the outer leaves. Early in the incipiency of the disease the marbling is in both cases very finely shaded. The ramifications of the veins contrast as a fine network against the light-colored tissue. In the "Black wood-vessel disease," this chlorosis is mostly followed by withering of the outer leaves, which was not observed in the plants suffering from copper deficiency. For these plants the transport of water to the leaves can take place without disturbance, as the wood vessels in the roots do not degenerate nor do they assume a dark color, as in the case of the black wood-vessel disease. In water cultures the supply of water to the plant is, however, particularly ideal, and it is evident that copper-deficient beets in the field show symptoms of earlier withering, owing to severe drought, than do sound beets, particularly if it also appears in the field that the relation of weight of foliage to weight of roots is larger for diseased plants than for sound ones.

Furthermore, it is quite possible, even probable, that plants with a root system weakened by copper deficiency, which is also long and thin, are much more sensitive to Pythium and other root parasites than plants that dispose of sufficient elements.

In the water cultures the dead leaf tissue showed finally a brown greyish, greyish, sometimes white, color. It is not unlikely that under natural conditions, whereby the plants are exposed to rain, the dead parts may finally become dark brown and (or) black in reacting to microorganisms, as is the case of the yellowing disease (32). Thorough field observations should reveal in what respects the symptoms of copper deficiency differ in practice from those induced in water culture.

The provisional impression is, on basis of symptoms of copper deficiency in water cultures compared with manganese-deficiency—simultaneously created in water cultures—that the principal points of difference between the two diseases are the following:—

(1) When there is copper deficiency the shading of the marbling is finer than in the case of manganese deficiency.
(2) In case of manganese deficiency the plants, in general, impress one as being more yellow.
(3) In case of manganese deficiency the leaf margins of the diseased leaves are generally more strongly curled in an upward direction.

As regards copper deficiency in other plants, it can be stated that Wilson and Townsend (47) described a chlorosis in lettuce that could be corrected by the application of copper salts. The disease occurred with a pH of 4.8.

Disease symptoms in fruit trees that could be corrected by application of copper sulphate have frequently been described as "exanthema" or "die back." The disease is especially known to affect citrus in Florida, as early as 1875. Since then this disease has been found in California, Cuba, Puerto Rico, Yucatan, Dutch Guinea, Brazil, Hawaii, Italy, Australia, the Philippines, and South Africa. A chlorosis in fruit trees caused by copper deficiency in South Africa was described by Anderssen (1) and Isaac (16). Anderssen states that the disease occurs on light sandy soils with a pH of 5.5–6.5. Plums, peaches, apricots, apples and pears react with chlorotic symptoms and death of the leaves and tips of the shoots. Isaac states that the lower leaves especially are affected and that application of calcium promotes the disease. *Vide* for further literature, Jacks and Scherbatoff (17). The trees may be cured by (a) application of copper salts to the soil in the neighbourhood of the bole, (b) spraying the trees with Bordeaux mixture and (c) also by putting copper salts into the bole.

Though copper deficiency may occur on alkaline soils, this disease is mostly found to occur on sour soils; this in contrast with manganese and iron deficiency. Also, boron deficiency occurs mostly (however, not always) on soils with an alkaline reaction. Liming, however, promotes copper deficiency in most cases; the same applies to manganese, iron and boron deficiency.

These four deficiency diseases will, moreover, when lime is applied, appear to be more pronounced in plants that grow best with an alkaline reaction. Firstly, these elements will be converted in such a soil condition into a less good soluble form; and secondly, relatively more of these elements will be necessary at a certain stage of growth, owing to the stronger growth of the plant caused by the high pH, than would be the case under a lower pH. This applies, therefore, particularly, to beets, peas, and beans.

Experiments of Sommer (43) and Lipman and McKinney (23) showed convincingly for the first time that plants demand copper for their normal development. Various investigations demonstrated that the growth of lower organisms (grey mould, yeast, algae, and bacteria) are greatly stimulated by slight quantities of copper (30, 6, 25, 34).

Copper probably plays a rôle in the oxidization processes, and it is not impossible that it also exercises an influence on certain vitamins. Schrenk (36) found an increase of oxidizing enzymes in kale sprayed with copper ammonium carbonate, whilst McHargue and Calfee (24) indicate that they found a relation between the occurrence of copper and vitamin A.

According to a statement of Scharrer and Schropp (35), Wieland (46) should have proved that copper has a catalytic reaction in the oxidizing processes, whilst Wertheimer (45) found that the oxidization of phenyleendiamin + $\alpha$ Naphtol to the pigment Indolphenol blue was caused by catalytic reaction of copper.

It is very probable that the function of copper in the plant and that in animals are analogical. The above investigation has shown that copper has an essential function in the formation and action of chlorophyll, as is probably the case with hemoglobine.

## LITERATURE CITED


1. ANDERSSEN, F. G. Chlorosis of deciduous fruit trees due to a copper deficiency. Jour. Pomol. and Hort. Sci. **10**: 130–146. 1932.
2. BRANDENBURG, E. Onderzoekingen over ontginningsziekte [I]–II, Tijdschr. Plantenziekten **37**: 17–48; **39**: 189–192. 1931–1933.
3. ————————. Ontginningsziekte en kopergebrek. Meded. Inst. Suikerbietent, Bergen op Zoom, **5**: 245–256. 1935.
4. CLAUSEN, H. Weisseuche oder Urbarmachungskrankheit. Mitt. Deut. Landw. Gesell. **49**: 919–920. 1934.
5. ELEMA, J. Ziekte in de haver op heideveldontginningen. Drentsch Landbouwblad. 1920.
6. ELVEHJEM, C. A. The rôle of iron and copper in the growth and metabolism of yeast. Jour. Biol. Chem. **90**: 111–132. 1931.
7. ————————, and E. B. HART. The relation of iron and copper to hemoglobin synthesis in the chick. Jour. Biol. Chem. **84**: 131–141. 1929.
8. ————————. The necessity of copper as a supplement to iron for hemoglobin formation in the pig. Jour. Biol. Chem. **95**: 363–370. 1932.
9. ————————, and W. C. SHERMAN. The action of copper in iron metabolism. Jour. Biol. Chem. **98**: 309–319. 1932.
10. FLINN, F. B., and J. H. INOUYE. Some physiological aspects of copper in the organism. Jour. Biol. Chem. **84**: 101–114. 1929.
11. HART, E. B., H. E. STEENBOCK, J. WADDELL, and C. A. ELVEHJEM. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem. **77**: 797–812. 1928.
12. HUDIG, J., and C. MEIJER. De ontginningsziekte en haar bestrijding. (3e Mededeeling) Ber. Rijkslandbouwproefst. Groningen. 2-afd. No. 18. 1925.
13. ————————. Kopersulfaat als vermoedelijk bestrijdingsmiddel tegen de ontginningsziekte. Ber. Rijkslandbouwproefst. Groningen. No. 19. 1925.
14. ————————. De Aanwending van kopersulfaat ter bestrijding van de ontginningsziekte. Ber. Rijkslandbouwproefst. Groningen. 2. afd. No. 22. 1925.
15. ————————. Über die sogenannte "Urbarmachungskrankheit" als dritte Bodenkrankheit. Ztschr. Pflanzenernähr. u. Düngung, Teil A, **8**: 14–52. 1926.

16. ISAAC, W. E. Researches on the chlorosis of deciduous fruit trees II. Experiments on chlorosis of peach trees. Roy. Soc. So. Africa, Trans. 22: 187–204. 1934.
17. JACKS, G. V., and H. SCHERBATOFF. Soil deficiencies and plant diseases. [Gt. Brit.] Imp. Bur. Soil Sci., Tech. Commun. 31. 1934.
18. KEIL, H. L., and V. E. NELSON. Rôle of copper in hemoglobin formation. Soc. Expt. Biol. and Med. Proc. 28: 392–393. 1931.
19. ————. The rôle of copper in hemoglobin generation and in reproduction. Jour. Biol. Chem. 93: 49–57. 1931.
20. ————. The rôle of copper in hemoglobin regeneration. Proc. Iowa Acad. Sci. 39: 163–7. 1932.
21. KRAUSS, W. E. Studies on the nutritive value of milk, I, II. Jour. Dairy Sci., 12: 74–79, 242–251. 1929.
22. LEWIS, G. T., T. E. WEICHSELBAUM, and J. L. MCGHEE. Effects of metals purified by electrolytic deposition on hemoglobin regeneration in anaemic white rat. Soc. Expt. Biol. and Med. Proc. 27: 329–331. 1930.
23. LIPMAN, C. B., and G. MACKINNEY. Proof of the essential nature of copper for higher green plants. Plant Physiol. 6: 593–599. 1931.
24. MCHARGUE, J. S. The association of copper with substances containing the fat-soluble A Vitamin. Amer. Jour. Physiol. 72: 583–594. 1925.
25. ————, and R. K. CALFEE. Effect of manganese, copper, and zinc on growth and metabolism of *Aspergillus flavus* and *Rhizopus nigricans*. Bot. Gaz. 91: 183–193. 1931.
26. ————, D. J. HEALY, and E. S. HILL. The relation of copper to the hemoglobin content of rat blood. Preliminary report. Jour. Biol. Chem. 78: 637–641. 1928.
27. MEIJER, C., EN J. HUDIG. De ontginningsziekte en haar bestrijding door stadscompost. de Veldbode. 34: 1924.
28. MEIJER, C. Verslag over een achttiental bemestingsproeven met kopersulfaat. Verslagen van Landbouwkundige onderzoekingen (Rijkslandb. Proefst. Groningen). No. 40. A., p. 67–173. 1934.
29. MUNTWYLER, E., and R. F. HANZA. Action of copper and other elements in iron metabolism. Soc. Expt. Biol. and Med. Proc. 30: 845–846. 1933.
30. ONO, N. Ueber die Wachsthumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Col. Sci. Imp. Univ. Tokyo. 13: 141–186. 1900.
31. QUANJER, H. M. Enkele kenmerken der "vergelings" -ziekte van suiker-en voederbieten ter onderscheiding van de "zwarte houtvaten"-ziekte. Tijdschr. Plantenziekten. 40: 201–214. 1934.
32. RADEMACHER, B. Praktische möglichkeiten zur Verhütung und Bekämpfung der Urbarmachungskrankheit. Fortschr. Landw. 7: 457–461. 1932.
33. ————. Die Heidemoorkrankheit (Urbarmachungskrankheit). Flügbl. Biol. Reichsanst. Land-u. Forstw. 137. 1935.
34. RUSSELL, R., and T. F. MANNS. The value of copper sulphate as a plant nutrient. Peninsula Hort. Soc. Proc. 47: 51–57. 1933.
35. SCHARRER, K., und W. SCHROPP. Sand- und Wasserkulturversuche über die Wirkung des Kupfer -ions. Ztschr. Planzenernähr. Düngung u. Bodenk., A. 32: 184–200. 1933.
36. SCHRENK, H. VON. Intumescences formed as a result of chemical stimulation. Missouri Bot. Gard. Ann. Rept. 16: 125–148. 1905.
37. SCHREVEN, D. A. VAN. De Vergelingsziekte bij de biet en haar Oorzaak. Meded. Inst. Suikerbietent., Bergen op Zoom. (French summary) 6: 1–36. 1936.

38. SCHREVEN, D. A. VAN. Kopergebrek bij de suikerbeit. Meded. Inst. Suikerbietent., Bergen op -Zoom. 6: 37–57. (French summary.) 1936.
39. ————————. Le manque de cuivre chez la betterave sucrière. Compte Rendu Définitif de la VI Assemblée de l'Institut International de Recherches Betteravières, réuni a Bruxelles les, 6, 7 et 8 Janvier 1936.
40. SJOLLEMA, B. Likzucht en ontginningsziekte. Landbouwk. Tijdschr. 45: 1933.
41. SMITH, W. S. Een Onderzoek naar het voorkomen en de oorzaken van de verschijnselen, welke worden aangeduid met den naam "Ontginningsziekte" 149 pp. Fa H. Veenman & Zonen, Wageningen. 1927.
42. SMITH, R. E., and H. E. THOMAS. Copper sulphate as a remedy for exanthema in prunes, apples, pears and olives. Phytopath. 18: 449–454. 1928.
43. SOMMER, A. L. Copper as an essential for plant growth. Plant Physiol. 6: 339–345. 1931.
44. STEINBERG, R. A. Nutrient-solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. Journ. Agr. Res. 51: 413–424. 1935.
45. WERTHEIMER, E. Ein antoxydables system als Modell einer Schwermetallkatalyse. Fermentforschung 8: 497–517. 1926.
46. WIELAND, H. Ueber den Mechanismus der Oxydationsvorgange VI. Liebigs. Ann. Chem. 434: 185–203. 1923.
47. WILSON, B. D., and G. R. TOWNSEND. Correction of the unproductivity of a peat soil for lettuce. Jour. Amer. Soc. Agron. 25: 523–527. 1933.

# THE LONGEVITY OF SMUT SPORES IN HERBARIUM SPECIMENS[1]

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## INTRODUCTION

It has long been known, in a general way, that the spores of some smut fungi possess considerable longevity when stored under conditions of moderate temperature and low relative humidity. This conception is based chiefly on fragmentary records of about 75 years.

In 1879 von Liebenberg, according to de Bary (1), indicated the viability of the spores of several smut fungi, including a demonstration of the longevity of $7\frac{1}{2}$ years for *Ustilago carbo*, (according to McAlpine (3) this record refers to *U. avenae* (Pers.) Jens.); $5\frac{1}{2}$ years for *U. kolaczekii* Kühn and *U. crameri* Körn.; $3\frac{1}{2}$ years for *U. rabenhorstiana* Kühn; $6\frac{1}{2}$ years for *Urocystis occulta* (Wallr.) Rab. and *Sphacelotheca sorghi* (Lk.) Clint.; and $8\frac{1}{2}$ years for *Tilletia tritici* (Bjerk.) Wint. de Bary (1) pointed out that since this record of *T. tritici* representd the oldest material von Liebenberg had at his command, and since the germination was so vigorous, it might be assumed that still older spores would have shown good germination. According to de Bary (1), Brefeld, as early as 1883 showed that the spores of *Sorosporium reilianum* (Kühn) McAlpine could retain their viability for 8 years.

McAlpine (3) has added several records of the longevity of smut spores: *Tolyposporium bursum* (Berk.) McAlpine 4 years; *Ustilago readeri* Sydow 2 years; *U. bromivora* (Tul.) F. d. W. at least $2\frac{1}{2}$ years; *U. cynodontis* Hem. at least 1 year; *Sorosporium consanguineum* Ell. and Ev. 3 years; and *Cintractia densa* McAlpine almost 5 years.

The foregoing longevity records have been supplemented in more recent years, by more or less scattered reports from various investigators, especially of the cereal smuts. Most of these reports have been incidental to other phases of research on smut fungi. Sobel (8) reports a longevity of 4 years for *Sphacelotheca cruenta*, but only 2 years for *S. sorghi*. Novak (5) reports almost unimpaired viability for *Tilletia levis* Kühn and *T. tritici* over a period of several years (exact number not given). Sobel (8) obtained germination of 7-year-old spores of *Tilletia tritici*, and Woolman and

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Humphrey (10) have shown that the spores of both species of *Tilletia* can retain their viability for 12 years or more.

Verwoerd (9) found that the spores of *Urocystis tritici* Körn. remain viable at least 5 years. Noble (4) augmented this record by obtaining vigorous germination of 10-year-old spores.

Spores of the oat-smut fungi likewise have been shown to possess considerable longevity. Sampson (7) reported only slight loss of viability for *Ustilago avenae* after 2 years; while Sobel (8) found that occasional spores 9½-years old would germinate. The records would indicate a greater longevity for *U. levis* (K. and S.) Magn. than for *U. avenae*. Thus, Sampson (7) found still viable the spores of a collection of *U. levis*, 5½ years old. Sobel (8) raised this record for *U. levis* to 13½ years.

According to Rump (6), *Ustilago hordei* (Pers.) K. and S. spores were still viable after 5 years, and Sobel (8) reported at least 7 years longevity for this species. *U. nuda* (Jens.) K. and S., on the other hand, seemed to be comparatively short-lived. Zeiner (11) reported germination of spores only 1 year old.

Benigni (2) was unable to obtain germination of the spores of *Ustilago zeae* (Beckm.) Unger older than 3 years.

Incidental to examining herbarium specimens of various smut fungi, the writer observed that a few collections that were several years old, contained a fair percentage of viable spores. Interest in the longevity of smut spores was thus aroused, and a survey of the literature revealed the fragmentary records presented above. It was then decided to make a concentrated study of the longevity of the spores of specimens of the Ustilaginales in the plant pathology herbarium of the State College of Washington, in an effort to supplement the above records with more exhaustive data.

## MATERIALS AND METHODS

With 2 or 3 exceptions (representing relatively recent collections) the data obtained from the viability tests in the present investigation were taken from herbarium specimens. Presumably, such specimens are authentic as to identity and date of collection, and should serve as reliable material to establish age limits of spore viability. Using poured plates, (Petri dishes), the spores of the Ustilaginaceae were tested on potato-dextrose agar, while those of the Tilletiaceae were tested on plain agar. As a precaution against including foreign smut spores in any test, spore material was taken from unbroken sori with a sterile needle or loop. In order to avoid removing the covers and to minimize the danger of contamination with other fungi, the plates were inverted and the spores examined through the bottom. Of the available specimens 387 collections were selected for tests, and of these, 80 were found to contain viable spores.

RESULTS

The results are presented in tabular form. Table 1 includes a list of the species tested and the ages of the specimens. The ages of specimens containing viable spores are in bold-face type. The numerous duplications are not shown.

The data obtained from the 80 specimens showing germination are presented in tables 2 and 3. The source of each specimen also is shown.

Following are the species of smut fungi tested for spore viability. The figures represent the age of the various specimens; those in bold-face type represent the age of specimens containing viable spores. USTILAGINACEAE: *Cintractia angulata* Syd. 12; *C. caricis* (Pers.) Magn. 34, 12, 8, 5, 4, **4, 3**; C. *junci* (Schw.) Trel. 28, 26, 24, **3**; *C. luzulae* (Sacc.) Clint. 11, 3; *Melanopsichum austro-americanum* (Speg.) G. Beck 29; *Schizonella melanogramma* (DC.) Schröt. 25; *Sorosporium melandrii* Syd. **2**; *S. ellisii* Wint. 28; *S. everhartii* Ell. and Gall. 27; *S. reilianum* (Kühn) McAlpine 38, 30, 16, **2**; *S. saponariae* Rudolphi 16, 12; *S. syntherismae* (Peck) Farl. 33, 24, 9, 8, **7, 6, 5, 4, 3**; *Sphacelotheca andropogonis* (Opiz) Bubak 11, 6; *S. hydropiperis* (Schum.) de Bary 50, 34, 11, 10, 9, 5, 4; *Sphacelotheca ischmaei* (Fkl.) Clint. 35, 5; *S. occidentalis* Seym. 25; *S. sorghi* (Lk.) Clint. 38, 25, 14, **13,** 9, 7; *Thecaphora cuneata* (Schof.) Clint. 26; *T. deformans* Dur. and Mont. 20; *T. trailii* Cke. 30; *Tolyposporium bullatum* Schröt. 36, 3; *T. junci* (Schröt.) Woronin 16; *T. leptideum* Syd. 10; *Ustilago anomale* J. Kunze 42, 11, 10, 4, 3; *U. aristidae* Pk. 35, 34; *U. avenae* (Pers.) Jens. 26, 22, 17, 16, 14, **13, 12,** 7, **4**; *U. betonicae* Berk. 25, 10; *U. bromivora* (Tul.) F. d. W. 43, 32, 30, 14, 12, **10,** 9, **7,** 4, **2**; *U. claytoniae* Shear 9; *U. crameri* Körn. 40, 37, 16, 9, **4**; *U. cynodontis* Hem. **5**; *U. echinata* Schröt. 13; *U. grandis* Fries. 12; *U. hordei* (Pers.) K. and S. 24, **23,** 22, **17, 16, 14, 13, 11, 7, 5, 3, 2**; *U. hypodites* (Schl.) Fries 47, 34, 13, 9, 7; *U. levis* (K. and S.) Magnus 39, 32, 24, 23, 17, 9, **4**; *U. longissima* (Sow.) Tul. 13, 11; *U. lorentziana* Thüm. 31, 24, 23, 15, 9, **3**; *U. major* 13, 11; *U. marginalis* (DC.) Lév. 1; *U. neglecta* Niessl. 46, 26, 9, **7, 6, 5, 4, 3**; *U. oxalidis* E. and T. 28, 7, 6, 5, 4, 3; *U. perennans* Rostr. 37, 16, 14, 12; *U. pustulata* (DC.) Wint. 10; *U. nuda* (Jens.) K. and S. 37, 25, 17, 7, 4; *U. rabenhorstiana* Kühn 45, 23, 12, **8, 7, 5, 4, 3**; *U. residua* Clint. 6; *U. striaeformis* (West.) Niesl. 16, 11, 9, 5, 4, 3; *U. succisae* P. Magnus 9; *U. tritici* (Pers.) Rostr. 25, 20, 16, 11, 6, 4; *U. utriculosa* (Nees) Tul. 28, 14, 11, 10, 7, 6, 5, 4; *U. warmingii* Rostr. 3; *U. violaceae* (Pers.) Fkl. 16, 11, 10; *U. zeae* (Beckm.) Unger 41, 27, 14, 9, **2**. TILLETIACEAE: *Entyloma calendulae* (Oud.) de Bary 11; *E. dahliae* Sydow **10**; *E. eryngii* (Cke.) de Bary **11**; *E. irregulare* Johans. 5; *E. lineatum* (Cke.) Davis 10; *E. physalidis* Wint. 10; *Doassansia alismatis* (Nees) Cornu 11; *D. saggitariae* (West.) Fisch. 11; *Tilletia anthoxanthi* Blytt 5, 4; *T. asperi-*

TABLE 1.—*Summary of successful germination tests of smut spores—Ustilaginaceae*

| Species | Place of collection | Age of spores yrs. | Approximate percentage of germination on potato-dextrose agar after no. days indicated: | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 day | 2 days | 4 days | 7 days | 12 days |
| Cintractia caricis | Mehoopany, Pa. | 4 | 0 | 0 | 0 | 0.1 | – |
| " " | Sweden, near Storlien | 3 | 0 | 0 | 0.1 | 0.1 | 0.1 |
| " " | " " " | 3 | 0 | 0 | 0.1 | 0.1 | 0.1 |
| Cintractia junci | Scotia, Pa. | 3 | 0 | 0 | 5.0 | 8.0 | 12.0 |
| Sorosporium melandrii | Germany, Rheinprovinz: Köln-Zollstock | 2 | 0 | 1.0 | 10.0 | 10.0 | – |
| Sorosporium reilianum | Pullman, Wash. | 2 | 0 | 45.0 | * | * | – |
| Sorosporium syntherismae | Chambersburg, Pa. | 7 | 0 | 0 | 0.1 | 0.1 | – |
| " " | Lewistown, Pa. | 7 | 0 | 0 | 0 | 1.0 | – |
| " " | Williamsport, Pa. | 6 | 0 | 10.0 | 30.0 | * | – |
| " " | Sunbury, Pa. | 6 | 0 | 0 | 0.1 | 1.0 | – |
| " " | Tunkhannock, Pa. | 5 | 0 | 0 | 8.0 | * | – |
| " " | Milford, Pa. | 5 | 0 | 1.0 | 40.0 | 50.0 | * |
| " " | Sunbury, Pa. | 4 | 0 | 2.0 | 5.0 | * | – |
| " " | Lock Haven, Pa. | 3 | 0 | 1.0 | 20.0 | * | – |
| Sphacelotheca sorghi | Daisy, Wash. | 13 | 10.0 | 40.0 | * | * | – |
| " " | State College, Pa. | 7 | 0 | 0 | 0.1 | 0.5 | – |
| Ustilago avenae | Puyallup, Wash. | 13 | 0 | 0 | 5.0 | 10.0 | * |
| " " | Germany, Rheinprovinz: Ruwer bei Trier | 12 | 0 | 0 | 0 | 0.1 | – |
| " " | Richardsville, Pa. | 4 | 0 | 0.1 | 0.2 | 0.5 | * |
| " " | Milheim, Pa. | 4 | 0 | 0.1 | 0.5 | * | * |
| " " | Centre Hall, Pa. | 4 | 0.5 | 15.0 | 25.0 | * | * |

TABLE 1.—*Summary of successful germination tests of smut spores—Ustilaginaceae—(Continued)*

| Species | Place of collection | Age of spores yrs. | Approximate percentage of germination on potato-dextrose agar after no. days indicated: | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 day | 2 days | 4 days | 7 days | 12 days |
| Ustilago bromivora | Waterville, Wash. | 10 | 40.0 | 50.0 | 75.0 | * | * |
| " " | Walla Walla, Wash. | 10 | 85.0 | 95.0 | * | * | – |
| " " | Munich, Bavaria | 7 | 0 | 0 | 0.1 | 0.5 | 1.0 |
| " " | Pullman, Wash. | 2 | 70.0 | 70.0 | 90.0 | * | – |
| " " | " " | 2 | 60.0 | 60.0 | 90.0 | * | – |
| Ustilago crameri | State College, Pa. | 4 | 0 | 95.0 | * | * | – |
| Ustilago cynodontis | Temple, Texas | 5 | 0 | 0 | 0.2 | 5.0 | * |
| " " | " " | 5 | 0.01 | 0.5 | 10.0 | * | – |
| Ustilago hordei | Bozeman, Mont. | 23 | 0 | 0 | 0 | 0 | 1.0 |
| " " | Puyallup, Wash. | 17 | 0 | 18.0 | 50.0 | * | * |
| " " | Vancouver, Wash. | 16 | 0 | 0.1 | 20.0 | – | – |
| " " | Monroe, Wash. | 14 | 0 | 0 | 0.5 | 1.0 | – |
| " " | Germany, Rheinprovinz: Casel bei Trier | 13 | 0.5 | 1.0 | 2.0 | 10.0 | * |
| " " | Pullman, Wash. | 11 | 90.0 | * | * | – | – |
| " " | Wilcox, Pa. | 7 | 45.0 | 75.0 | 85.0 | * | – |
| " " | Hyde, Pa. | 7 | 80.0 | 95.0 | * | * | – |
| " " | St. Marys, Pa. | 5 | 35.0 | 90.0 | * | – | – |
| " " | Ridgway, Pa. | 5 | 45.0 | 60.0 | * | * | – |
| " " | Pullman, Wash. | 3 | 80.0 | 95.0 | * | – | – |
| " " | " " | 2 | 95.0 | * | * | – | – |
| Ustilago levis | Kylertown, Pa. | 4 | 0.5 | 1.0 | 1.0 | 10.0 | * |
| " " | Bellefonte, Pa. | 4 | 10.0 | 40.0 | 50.0 | * | – |

TABLE 1.—*Summary of successful germination tests of smut spores—Ustilaginaceae—(Continued)*

| Species | Place of collection | Age of spores yrs. | Approximate percentage of germination on potato-dextrose agar after no. days indicated: | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 day | 2 days | 4 days | 7 days | 12 days |
| Ustilago lorentziana | Salmon, Idaho | 3 | 50.0 | * | * | – | – |
| Ustilago mulfordiana | Kettle Falls, Wash. | 9 | 0 | 0 | 0 | 60.0 | * |
| " " | " " " | 9 | 0 | 0 | 0 | 0.5 | 0.5 |
| Ustilago neglecta | Beaver Falls, Pa. | 7 | 0 | 0 | 0 | 0.1 | 0.5 |
| " " | Youngsville, Pa. | 7 | 0 | 0 | 0 | 0 | 30.0 |
| " " | Orange, Pa. | 7 | 0 | 0 | 0 | 0 | 0.1 |
| " " | State College, Pa. | 6 | 0 | 0 | 0 | 0.1 | – |
| " " | North East, Pa. | 6 | 0 | 0 | 0 | 0 | 0.2 |
| " " | Wayne Co., Pa. | 5 | 0 | 0 | 60.0 | 75.0 | * |
| " " | Potter Co., Pa. | 4 | 0 | 0 | 40.0 | 80.0 | * |
| " " | Burgettstown, Pa. | 3 | 0 | 0 | 0 | 90.0 | * |
| " " | St. Lawrence, Pa. | 3 | 0 | 0 | 90.0 | * | – |
| Ustilago rabenhorstiana | Winsor, Conn. | 8 | 0 | 0 | 0 | 0.1 | – |
| " " | Monaca, Pa. | 7 | 0 | 0.1 | 1.0 | 1.0 | – |
| " " | Mehoopany, Pa. | 5 | 0 | 0 | 5.0 | 40.0 | * |
| " " | Bowmansdale, Pa. | 5 | 0 | 0 | 1.0 | 1.0 | * |
| " " | Jamestown, Pa. | 4 | 0 | 0 | 0.1 | 60.0 | * |
| " " | State College, Pa. | 3 | 0 | 0 | 80.0 | * | – |
| Ustilago zeae | Pullman, Wash. | 2 | 90.0 | * | – | – | – |

* Impossible to determine any increase in percentage of spore germination because of the maze of mycelium, or bud sporidia or both. On week-old plates contaminating fungi often hindered observations.

– Not examined further.

TABLE 2.—*Summary of successful germination tests of smut spores—Tilletiaceae*

| Species | Place of collection | Age of spores yrs. | Approximate percentage of germination on plain agar after no. days indicated: | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 day | 2 days | 4 days | 7 days | 12 days | 21 days | 30 days |
| Entyloma dahliae | Munich Botanic Gard. | 10 | 0 | 0 | 0 | 1.0 | 8.0 | 10.0 | – |
| Entyloma eryngii | Germany: Cuxhaven, Hamburg | 11 | 0 | 0 | 0 | 5.0 | 25.0 | 25.0 | * |
| Tilletia levis | Stockton, Kansas | 25 | 0 | 0 | 0 | 0 | 5.0 | 20.0 | * |
| " " | Guelph, Ont. | 18 | 0 | 0 | 0 | 1.0 | 75.0 | * | * |
| " " | Beaver, Utah | 17 | 0 | 0 | 0 | 0 | 2.0 | 50.0 | * |
| " " | Lewisburg, Pa. | 6 | 0 | 0 | 0 | 5.0 | 60.0 | * | * |
| " " | Fogelsville, Pa. | 4 | 0 | 0 | 1.0 | 95.0 | * | * | – |
| Tilletia rauwenhoffii | Brandenburg, Germany | 12 | 0 | 0 | 0 | 0 | 0 | 7.0 | – |
| Tilletia separata | Moscow, Russia | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 10.0 |
| Tilletia tritici | Coupeville, Wash. | 18 | 0 | 0 | 0 | 5.0 | 60.0 | * | – |
| " " | Thornton, Wash. | 17 | 0 | 0 | 0 | 10.0 | 60.0 | * | – |
| " " | Goldendale, Wash. | 17 | 0 | 0 | 0 | 5.0 | 50.0 | 60.0 | * |
| " " | Pleasant Prairie, Wash. | 16 | 0 | 0 | 0 | 20.0 | 90.0 | * | * |
| " " | Ridgefield, Wash. | 16 | 0 | 0 | 0 | 1.0 | 80.0 | * | * |
| " " | Lowden, Wash. | 16 | 0 | 0 | 0 | 5.0 | 75.0 | * | * |
| " " | Port Angeles, Wash. | 16 | 0 | 0 | 0 | 1.0 | 30.0 | 60.0 | * |
| " " | Germany: Trier, Rheinprovinz | 13 | 0 | 0 | 0 | 0 | 40.0 | * | * |

* The maze of mycelium and germinating secondary sporidia prevented the determination of any increase in percentage of germination.

– Not examined further.

*folia* E. and E. 16, 15, 10, 9; *T. holci* (West.) Rostr. 17, 16, 10, 9, 7, 6, 5; *T. levis* Kühn 44, 42, **25,** 24, 23, **18, 17, 6, 4**; *T. guyotiana* Har. 16, 10; *T. rauwenhoffii* F. d. W. 13, **12,** 10, 9; *T. secalis* (Cda.) Kühn 12; *T. separata* J. Kunze **12**; *T. tritici* (Bjerk.) Wint. 42, **18, 17, 16, 13, 12**; *Tubercinia anemones* (Pers.) Liro 12; *T. cepulae* (Frost) Liro 12; *T. colchici* (Schlecht.) Liro 13; *T. ficariae* (Unger) Liro 12; *T. hellbori-viridis* (DC.) Liro 11; *T. hepaticae-trilobae* (DC.) Liro 12.

## DISCUSSION

An analysis of the data presented in the preceding paragraph and in tables 1 and 2 reveals a surprising spore longevity for some smut fungi. Of the 23 species showing viable spores from herbarium specimens, *Tilletia levis* possessed the greatest longevity. A collection made at Stockton, Kansas, 25 years ago, showed 20 per cent germination after 21 days. Several collections of *T. levis* and *T. tritici,* 16 to 18 years old, showed 50–75 per cent germination after 12 or more days.

While, in general, the greatest longevity seems to be possessed by species in the Tilletiaceae, certain of the Ustilaginaceae are capable of retaining their viability over remarkably long periods of time. The greatest distinction goes to *Ustilago hordei,* of which 16- and 17-year-old specimens showed 20 per cent and 50 per cent germination, respectively, after 4 days, while a 23-year-old specimen showed 1 per cent germination after 12 days.

The results of the present investigation establish a number of new records for spore longevity in the smut fungi. The greatest reported heretofore for *Tilletia levis* and *T. tritici* was 12 years (10). The data presented in this paper extend this to 25 years and 18 years for *T. levis* and *T. tritici,* respectively. The greatest longevity established heretofore for *Ustilago hordei* was 7 years (8); the present data show that this species can retain slight viability for 23 years. Sobel (8) established the longevity of *U. avenae* at 9½ years; in the present investigation a 13-year-old specimen showed 10 per cent germination after 7 days. The same author (8) reported a retention of viability of only 2 years for *Sphacelotheca sorghi.* The writer obtained 40 per cent germination from a specimen 13 years old, and 0.5 per cent germination from one 7 years old.

On the other hand, for a few species, tests by the writer failed to equal the longevity records established by other investigators. Thus, both Sampson (7) and Sobel (8) have established a greater longevity for *Ustilago levis* than for *U. avenae.* The former obtained germination from collections 5½ years old, and the latter from a collection 13½ years old. The author was unable to obtain germination from collections more than 4 years old.

Spore longevity tests remind one again of the danger of introducing plant pathogens through herbarium specimens, when these are improperly

cared for after being received. Eleven of the 80 herbarium specimens showing viability came from foreign countries; of these 11 specimens, 7 are now 10 years or more old. Disinfection of herbarium specimens with carbon tetrachloride or carbon disulphide, and subsequent storage with napthaline crystals, apparently do not destroy the viability of smut spores.

Age certainly is not the only factor controlling the viability of the spores of the smut fungi. A collection of *Sphacelotheca sorghi* 13 years old showed 40 per cent germination after only 2 days, while another collection only 7 years old showed a maximum of only .5 per cent germination after 7 days. A collection of *Ustilago avenae* 13 years old gave 10 per cent spore germination after 7 days; another only 4 years old showed only .5 per cent after the same length of time. Two collections of *Ustilago bromivora,* 10 years of age, showed 75 per cent and 95 per cent germination, respectively, after only 4 days, while collections 9 and 4 years old showed no germination, even after 12 days on potato-dextrose agar. Also, of 5 specimens of *Tilletia tritici* 17 years old, 2 showed 50 to 60 per cent germination after 12 days, while the other 3 specimens showed no germination. Numerous other examples from the data could be cited to illustrate the point. Sampson (7) is of the opinion that for a given species (at least in the oats smuts) the state of maturity at the time of collection determines to a large extent the longevity of a given sample. *Within a species,* then, fully mature spores could be expected to retain their viability a much longer time than partially mature spores. The factors that cause the marked differences in spore longevity among the various species of smut fungi are not known and the present investigations throw no light upon this problem.

## SUMMARY

Three hundred and eighty-seven herbarium specimens of 77 species of smut fungi were tested for longevity of their spores.

Of these, 80 specimens, representing 24 species, were found to contain viable spores. Eleven of these specimens were from foreign countries.

Some species were found to retain their viability for a long time. Noteworthy among these are: *Tilletia levis,* 25 years; *T. tritici,* 18 years; *Ustilago hordei,* 23 years; *U. avenae,* 13 years; *Sphacelotheca sorghi,* 13 years; and *U. bromivora,* 10 years. These records are much higher than those established by previous investigators.

Considerable differences in viability were noticed in various collections of the same age and of the same species. Such differences are thought to be correlated with the degree of maturity of the spores at the time of collection.

In several instances collections 12 to 18 years old would show decidedly greater percentage of germination than much younger collections

of the same species. This would seem to lend support to Sampson's (7) opinion that *within a species* the state of maturity at the time of collection determines, in large measure, the longevity of the spores.

In general, the species belonging to the Tilletiaceae are possessed of greater longevity than those belonging to the Ustilaginaceae.

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LITERATURE CITED

1. BARY, A. DE. Comparative morphology and biology of the fungi, Mycetozoa and bacteria. 525 pp. Clarendon Press, Oxford. 1887.
2. BENIGNI, E. Note sull' *Ustilago maydis* (DC.) Corda nella Velle Padana. Riv. Patol. Veg. 17: 57–72. 1927. [Abstract in Rev. Appl. Mycol. 6: 546–547. 1927.]
3. MCALPINE, D. The smuts of Australia. 288 pp. J. Kemp, government printer, Melbourne. 1910.
4. NOBLE, R. J. Note on the longevity of spores of the fungus *Urocystis tritici* Koern. Jour. and Proc. Roy. Soc. New South Wales 67: 403–410. 1934.
5. NOVAK, S. K otazce infekce psenice výtrusy *Tilletia tritici* ruzného stari. Ochrana Rostlin 9: 30–32. 1929. [Abstract in Rev. Appl. Mycol. 8: 768. 1929.]
6. RUMP, L. Studien über den Gerstenhartbrand (*Ustilago hordei* Kell. u. Sw.). Forsch. Geb. Pflanzenkrank. Immunität. Pflanzenr. no. 2: 21–76. 1926. [Abstract in Rev. Appl. Mycol. 5: 604–605. 1926.]
7. SAMPSON, K. The biology of oat smuts. I. Viability of the chlamydospores. Ann. Appl. Biol. 15: 586–612. 1928.
8. SOBEL, M. The viability of the spores of the cereal smuts. Biologist 15: 95–96. 1933.
9. VERWOERD, L. The biology, parasitism and control of *Urocystis tritici* Koern., the causal organism of flag smut in wheat (*Triticum* spp.) and recording the occurrence of *Urocystis occulta* (Wallr.) Rab. in South Africa as the cause of "stem smut" in rye. South Africa Dept. Agr. Sci. Bull. 76. 1929. [Abstract in Rev. Appl. Mycol. 10: 371–372. 1931.]
10. WOOLMAN, H. M., and H. B. HUMPHREY. Studies in the physiology and control of bunt, or stinking smut, of wheat. U. S. Dept. Agr. Bull. 1239. 1924.
11. ZEINER, W. Das Verhalten verschiedener Sommergersten Kreuzungen hinsichtlich der Anfälligkeit für *Ustilago nuda*. Zschr. Zücht. Reihe A, Pflanzenzücht. 17: 229–264. 1932.

# ANGUILLULINA PRATENSIS IN RELATION TO ROOT INJURY OF APPLE AND OTHER FRUIT TREES

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(Accepted for publication March 16, 1936)

In the course of a study of dieback and rosette (little leaf (1)) of the apple, the fine feeding roots were found to be distinctly injured. The search for a specific cause revealed the presence (within the roots), in considerable number a nematode that was identified by Steiner as the meadow nematode, *Anguillulina pratensis* (De Man, 1880) Goffart, 1929, and reported by him as a new parasite for apple roots (6)[1]. More recently this nematode has been found in the roots of plum, peach, pear, and grapes, and in these cases also in association with the little leaf disease. The roots of fig trees, however, seem to be particularly susceptible to direct injury by this parasite (2, 8), although this fruit tree is seldom found affected by little leaf. Moreover, among the many hosts previously known for *A. pratensis* (5), many species are not known to be affected by little leaf or dieback. Nevertheless, the obvious direct injury to the roots and the possible secondary rôle of the nematodes in the production of the above diseases seem to merit further study. A different species of nematode, *Tylenchulus semipenetrans* Cobb 1913, was found some years ago (7) in the roots of citrus trees affected by mottle leaf which now seems to be similar in nature to the little leaf of deciduous fruit trees.

Early symptoms appear as necrotic spots on the fine white rootlets, ranging from microscopic size to streaks several millimeters in length and from dark amber to black (Fig. 1). These symptoms are very similar to those produced on pineapple (3) by the same (4, 5) or a very similar nematode. Heavily infested roots usually are stunted and sometimes thickened and distorted. Among infected roots are commonly found tufts, such as that shown in figure 1-D, that resulted from the repeated development of branches that are in turn killed back. It is not clear, however, that all of this type of symptom is to be charged to the activity of the nematodes. The larger roots of the apple and stone fruit trees that have been examined, unlike those of figure 1, exhibit no symptoms.

The nematodes invade only the cortex and align themselves more or less parallel with the axis of the rootlet. Eggs and larvae in all stages of development may be found together (Fig. 2).

[1] Steiner, G., and E. M. Buhrer. Observations on nematode diseases of plants. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reptr. 19 (3): 24–25. 1935. [Mimeographed.]

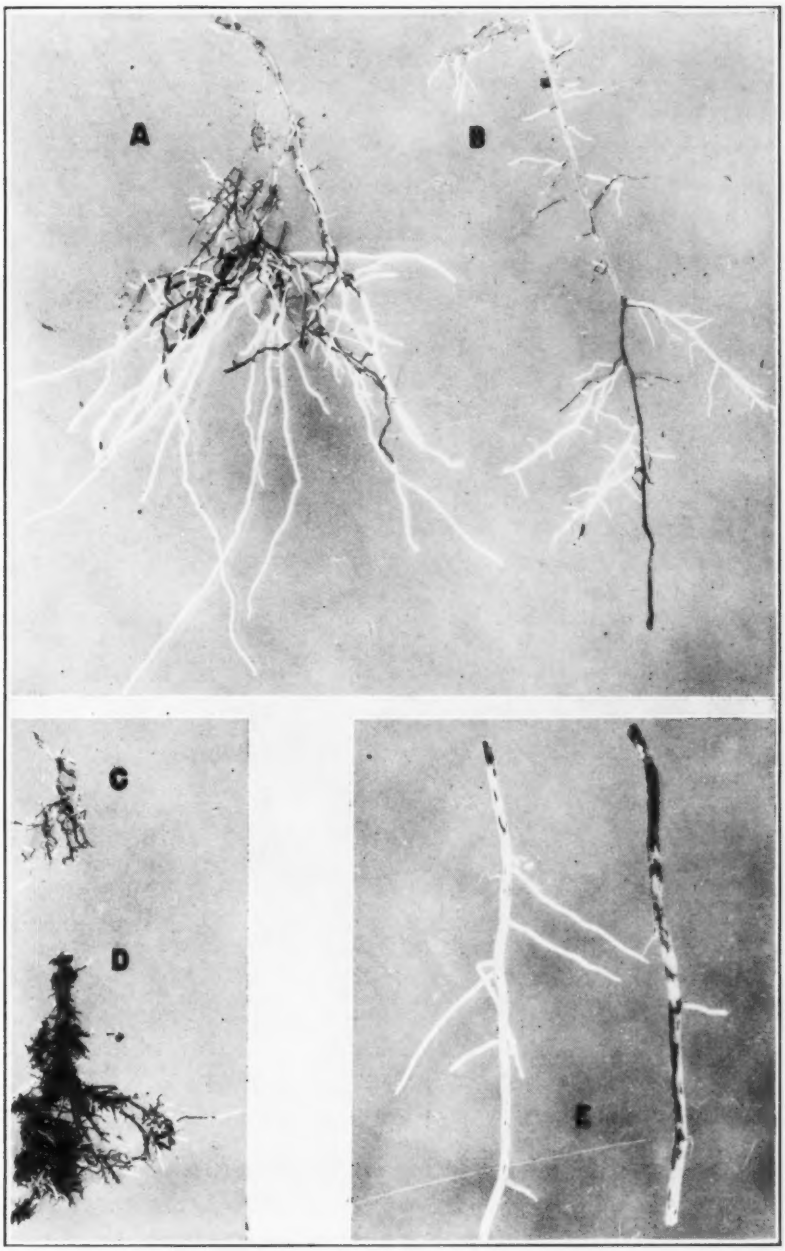


FIG. 1. A–B. Apple roots from a healthy orchard (Elphic's, Sonoma County). Note long, slender, widely spread healthy rootlets. C–E. Apple roots of trees with dieback symptoms (McKenzie's orchard, Sonoma County). In C and D note stunted condition and new rootlets of dwarfed type. In E note numerous blackish and dark brown lesions. These numerous black streaks may form large and sunken lesions. × approximately 1.

FIG. 2. Apple rootlets. A. Intracellular nematodes and their eggs prepared by modified Godfrey's method. B. Nematode and eggs in another root. × approximately 125.

In fixed material of apple rootlets stained with Flemming's triple stain, bacteria were found abundant, not only behind and in the immediate vicinity of the nematode, as has been noted by other workers (3) (8), but also to 10 or 15 cells distant from the nematode or its path. In the latter case the cells, by which the bacteria were surrounded, bore no evidence of disintegration. A few cells near the nematode were collapsed and filled with bacteria (Figs. 3, B and 4).

FIG. 3. A. Longitudinal section of a rootlet infested with nematodes. Section 8 μ thick and stained with the Gentian violet stain. Note the breaking of cell walls as nematode paved its way. × approximately 125. B. Longitudinal section through the cortex of a rootlet showing nests of nematodes and their eggs. Sections 8 μ thick and triple-stained. × approximately 125.

FIG. 4. A. Small portion of a section shown in figure 3, A. Abundance of bacteria in cells occupied by a nematode and also in many cells away from it. Sections 8 μ thick and triple-stained. B. Bacteria in cells infested with a nematode. Stoughton's method. Sections 8 μ thick. ×1000.

Two different species of bacteria were consistently associated with this nematode in infested roots. One species is identified as *Pseudomonas fluorescens* (Flügge) Migula, while the taxonomic position of the second has not been determined. The characters for the second organism are as follows: A short rod, motile by one polar flagellum, measuring 1 μ–1.9 μ × 0.6 μ–0.9 μ, nonsporiferous; Gram-negative, grows well in most of the common bacteriological media; it grows as grayish white film on beef-agar slants, the growth being more abundant on potato-dextrose (2 per cent)-peptone agar slants; it produces acid and gas in dextrose, sucrose, maltose, galactose, mannite, and arabinose; grows well in Fermi's solution; no growth in Cohn's and Uschinsky's solutions; gelatin liquefied; indol produced and nitrates reduced; no diastatic action on starch. It grows at 10° C., but not at 5° C., optimum for growth being 30° C. The upper thermal death point is 46° C.

The same organisms were obtained from apple roots experimentally infested with nematodes in the greenhouse. Inoculum was prepared by taking roots heavily infected with nematodes, washing and disinfecting them in a 1–1000 solution of $HgCl_2$ for 3 minutes, with subsequent washings in sterile distilled water, and teasing the tissues to free the nematode larvae into distilled water. This then was introduced into a sterilized soil with apple seedlings. It is impossible to conclude whether these bacteria are merely carried mechanically or associated in some more specific relation with the nematodes. Fifty apple seedlings grown in sterilized soil to which these species of bacteria were added separately or in combination in the absence of nematodes did not become diseased—their roots and tops remained normal.

In another experiment ten seedling apple trees were planted in each of the 3 following soils: soil heavily infested with *Anguillulina pratensis*, steam sterilized soil with nematodes, and the same kind of soil without the addition of nematodes. Plants in nonsterilized soil and sterilized soil plus nematodes, although lacking symptoms on leaves or stems, were decidedly less thrifty and made less growth than the plants in the sterilized soil without nematodes. All plants were fertilized with calcium nitrate.

The degree of injury caused by the nematode alone in the orchard, or even in the greenhouse, cannot be determined with certainty until methods are devised for freeing the nematodes from associated bacteria and other organisms. The conclusion seems inescapable, however, that a considerable amount of direct injury must follow the invasion of the feeding roots of fruit trees by large numbers of these nematodes.

A matter of possibly greater interest in relation to the particular orchards under consideration is the opening of avenues into the roots for the entrance of microorganisms, or for the absorption of preformed toxic materials from the organisms around the roots. That such toxic materials are present in the root zone has been demonstrated by the wilting of de-

tached apple shoots placed either in the filtered leachate of soil from around affected roots or in the filtrate from pure cultures of organisms isolated from the root zone of affected apple, apricot, and peach trees.

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LITERATURE CITED

1. CHANDLER, W. H., D. R. HOAGLAND, and P. L. HIBBARD. Little-leaf or rosette in fruit trees. Amer. Soc. Hort. Sci. Proc. **28** (1931): 556–560. 1932.
2. COBB, N. A. *Tylenchus penetrans* Cobb. Jour. Parasitol. **14**: 71. 1927.
3. GODFREY, G. H. A destructive root disease of pineapples and other plants due to *Tylenchus brachyurus*, n. sp. Phytopath. **19**: 611–29. 1929.
4. GOODEY, T. The genus Anguillulina Grev. and v. Ben., 1859, vel *Tylenchus* Bastian, 1865. Jour. Helminthol. **10**: 75–180. 1932.
5. ————. Plant parasitic nematodes and the diseases they cause. 306 pp. Methuen & Co., London. 1933.
6. STEINER, G. *Tylenchus pratensis* and various other nemas attacking plants. Jour. Agr. Res. [U. S.] **35**: 961–981. (1927) 1928.
7. THOMAS, E. E. The citrus nematode, *Tylenchulus semipenetrans*. California Agr. Expt. Sta. Tech. Paper 2: 1–19. 1923.
8. THORNE, G. Some plant parasitic nemas, with descriptions of three new species. Jour. Agr. Res. [U. S.] **49**: 755–769. 1934.

# POWDERY MILDEW (ERYSIPHE POLYGONI) ON GARDEN SNAP BEANS

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## INTRODUCTION

Powdery mildew (*Erysiphe polygoni* DC.) has been known for many years to attack both garden and field beans, but comparatively few workers have attached any particular importance to it. That the disease is widely distributed, however, is indicated in numerous references in the literature since 1889.

During the past 10 years, powdery mildew has become increasingly important throughout the southeastern part of the United States, particularly on the fall crop of beans, which is harvested during September, October, and November. In many cases the disease has caused a dwarfing of the plants, partial defoliation, and pod spotting, which has resulted in heavy losses to the growers. Since little or no systematic study had previously been devoted to the development and control of this malady, a series of field experiments to this end was started in 1931 and continued through 1935. These experiments were conducted near Charleston, South Carolina, at the substation of the State Agricultural Experiment Station, in a region largely devoted to the growing of vegetable crops for the market. It is believed that the data published here are applicable to the control of the disease in the territory included in the South Atlantic Seaboard.

## HISTORICAL

The first announcement of damage from powdery mildew on beans was from Bermuda in 1889, by Galloway.[3] Whetzel[4] reported it again from the same place in 1922, stating that it was the most important disease on beans at that time. Sherbakoff[5] noted a general infection in Florida in 1917, and also described briefly the pod spot that recently has become of considerable economic importance along the Atlantic Coast from Virginia southward.

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[2] Acknowledgment is made to Dr. L. L. Harter, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, for valuable suggestions during the course of this work and for criticisms of the manuscript.

[3] Galloway, B. T. Powdery mildew of the bean. Jour. Mycol. [U. S.] 5: 214. 1889.

[4] Whetzel, H. H. Report of the Pathologist. Bermuda Bd. and Dept. Agr. Repts. 1921: 41. 1922.

[5] Sherbakoff, C. D. Some important diseases of truck crops in Florida. Florida Agr. Expt. Sta. Bull. 139: 217–218. 1917.

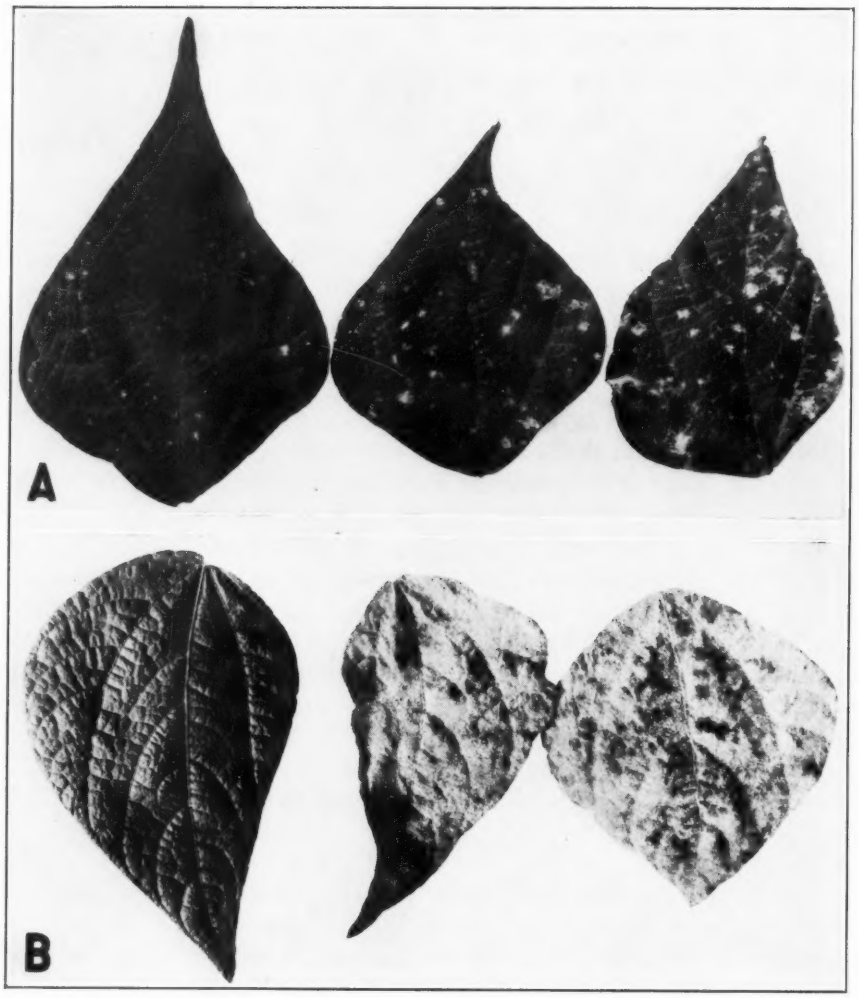


FIG. 1. A. Mature bean leaves showing initial stages of powdery mildew infection. Leaf on left shows spots about 1 day after first being visible. Those on right show spots 3 days old. B. Bean leaves showing advanced stage of infection. Note complete fungous covering of 2 diseased leaves on right as compared with healthy leaf on left.

Linford[6] reported powdery mildew causing a dwarfing of the young pods in Utah in 1927. Cook[7] described the powdery mildew pod spots as the

[6] Linford, M. B. Powdery mildew damages beans. U. S. Dept. Agr. Bur. Plant Indust. Plant Disease Rept. **11**: 137–138. 1927. [Mimeographed].

[7] Cook, H. T. Powdery mildew disease of snap beans. Virginia Truck Expt. Sta. Bull. 74. 1931; The control of powdery mildew of snap beans. Peninsular Hort. Soc. [Del.] Trans. **46**: (Delaware State Bd. Agr. Bull. V. **22** (5)): 25–27. 1932.

cause of heavy losses in Virginia in 1931. He described the disease, as it occurs in that section, in considerable detail and reported data on experimental control with both sulphur sprays and sulphur dusts. Numerous plant disease reports[8] indicate that powdery mildew has become of considerable importance over a large section of the bean-growing territory since 1920, causing appreciable losses in many instances.

## DESCRIPTION

The disease first appears on the upper surface of the older leaves as very small, white, circular, powdery spots (Fig. 1, A). These enlarge rapidly, coalesce to include larger areas, and finally cover the entire leaf (Fig. 1, B). In some instances infection may originate at points along the midrib or veins, the spots being circular, at first, then becoming elongate. The lower surface of the leaf may become infected at the same time as the upper, although this it not always the case. If infection occurs before the plants are mature, the leaves may become dwarfed, turn yellow, and finally fall off.

Fungus development on the stems and petioles is much the same in appearance as on the midribs and veins, although it usually follows foliage infection. The fungus grows rapidly over the surface of the leaves and, in advanced stages, the foliage appears to be covered with a talcum-like powder.

Powdery mildew may attack the immature pods just as it does the stems, causing serious dwarfing and premature death (Fig. 2, A). In such cases the fungus appears as a powdery coating over nearly all of the pod. It does not produce a distinct spot. This type of infection may be found in almost any generally infected field near the close of the harvest season but causes only slight losses, as a general rule. During certain seasons, and on the maturing pods, a more severe infection occurs, which begins as very small moist-looking circular spots. The spots enlarge rapidly, coalesce, and cause large irregular areas that may eventually cover the whole pod (Fig. 2, B). The early stages in the development of these spots show the powdery-appearing fungus growth, which soon becomes a light brown and finally a reddish brown. In severe cases the large spots may be slightly sunken.

## EXPERIMENTAL MATERIALS AND METHODS

Experimental plots, each 100 feet long and 4 rows wide, were used in this study. All treatments were replicated 3 or more times, the plots in all cases being systematically distributed over the experimental area. Data

[8] Tims, E. C. Powdery mildew (*Erysiphe polygoni*). U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reptr. 18: 94. 1934. [Mimeographed]. Barrus, M. F., O. C. Boyd and J. I. Wood. *Ibid.*, Sup. 81: 111. 1931. Miller, P. R., N. E. Stevens and J. I. Wood. *Ibid.*, 84: 32. 1933. Wood, J. I., N. E. Stevens and P. R. Miller. *Ibid.*, 85: 46. 1933.

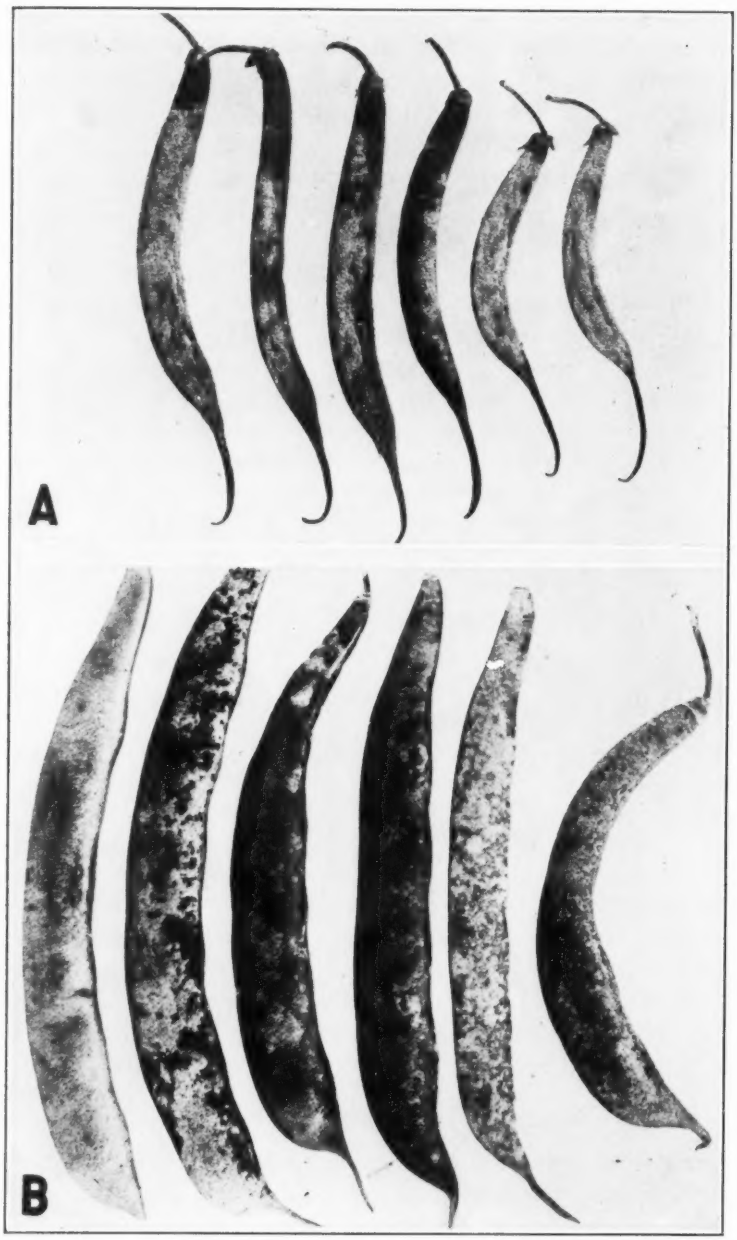


FIG. 2. A. Young bean pods showing heavy infection of powdery mildew. Note that the fungus had killed the immature pods. B Mature bean pods showing various stages of spotting caused by powdery mildew.

on both foliage and pod infections were collected only from the 2 center rows of each plot. Dust materials, consisting of various mixtures of sulphur and lime, sulphur and colloidal clay, different commercial sulphur dusts, and copper-lime dust, were applied with rotary hand dusters at the rate of 25 pounds per acre. All dust applications were made in late afternoon or early morning, when there was sufficient moisture on the foliage to insure a thorough sticking of the different materials. The spray materials, consisting of lime-sulphur spray, Bordeaux mixture, and two concentrations of flotation sulphur, were applied with hand-spray machines at the rate of approximately 100 gallons per acre. These applications were made when there was no dew on the foliage. The first application of both sprays and dusts was made when the initial stage of mildew infections appeared on the leaves and subsequent applications followed at approximately 10-day intervals, depending upon rainfall. In all cases the disease appeared so late in the growing season that not more than 2 applications of the most

TABLE 1.—*Combined data on powdery mildew foliage infection on beans when various dusts were used as controls in 1931 to 1935*

| Fungicide | Plants examined | Plants infected | Plants infected |
|---|---|---|---|
| Data for years 1931–1935 | | | |
| | *No.* | *No.* | *Per cent* |
| Nontreated check | 5490 | 4797 | 87.3 |
| Sulphur dust 100 per cent | 2382 | 54 | 2.2 |
| Sulphur-lime dust 75–25 | 2337 | 87 | 3.7 |
| Sulphur-lime dust 50–50 | 2250 | 139 | 6.1 |
| Sulphur-lime dust 25–75 | 2328 | 837 | 35.9 |
| Data for years 1931–1934 | | | |
| Nontreated check | 4690 | 3997 | 85.2 |
| Copper-lime dust 25–75 | 1816 | 777 | 42.7 |
| Data for 1933–1934 | | | |
| Nontreated check | 1418 | 1241 | 87.5 |
| Sulphur-clay 75–25 | 389 | 6 | 1.5 |
| Sulphur-clay 50–50 | 343 | 23 | 6.7 |
| Data for year 1932 | | | |
| Nontreated check | 800 | 800 | 100.0 |
| Manganar Rose dust 85 per cent sulphur | 400 | 41 | 10.2 |
| Koppers sulphur dust | 400 | 18 | 4.5 |
| Kolodust | 165 | 5 | 3.0 |
| Data for year 1933 | | | |
| Nontreated check | 1391 | 1391 | 100.0 |
| Koppers sulphur } 25–75 | 329 | 8 | 2.4 |
| Electric sulphur } | | | |
| Koppers sulphur } 50–50 | 316 | 0 | 0.0 |
| Electric sulphur } | | | |

effective materials were necessary to protect the plants throughout the harvest period.

Of the several fungicides tested in this work, only the sulphur-lime mixtures were used throughout the 5-year period. Some materials were too expensive to be of economic value to the growers, while others proved to be inefficient or of inadequate physical condition for a thorough covering of the plants. As these weaknesses appeared, the materials were discarded in favor of the more promising ones.

*Dust.* In table 1, it seems that essentially all dusts used, containing 75 per cent or more of sulphur, gave a high degree of control for powdery mildew. The Koppers sulphur-electric sulphur combination of 50 per cent of each was superior to any of the materials used, but the cost of this material was too great when its efficiency was compared with that of the 75–25 sulphur-lime combination. The sulphur-clay mixtures, while giving a high degree of control, were objectionable because of a tendency of the dust to clog the dust guns. Pure sulphur was difficult to use for the same reason. Of the several dust materials used, the sulphur-lime 75–25 combination proved to be the most desirable control for powdery mildew when the fungicidal action, ease of handling, and economy of the material are taken into consideration.

TABLE 2.—*Combined data on powdery mildew foliage infection of beans where different sprays were used as controls for the years 1931 to 1935*

| Fungicide | Plants examined | Plants infected | Plants infected |
|---|---|---|---|
| | *No.* | *No.* | *Per cent* |
| Nontreated check | 2218 | 2041 | 92.0 |
| Bordeaux mixture 4–4–50 | 1408 | 247 | 17.5 |
| Lime-sulphur spray | 1381 | 328 | 23.8 |
| Flotation sulphur spray 1–160 | 1332 | 80 | 6.0 |
| Flotation sulphur spray 1–320 | 975 | 131 | 13.4 |

*Sprays.* Of the four spray materials tested, the Flotation Sulphur 1–160 proved to be the most efficient (Table 2). Regular lime-sulphur (dry lime-sulphur 3–400) proved highly efficient in seasons of little or no rain, but did not give a good control during periods of intermittent rains. In no case did Bordeaux mixture give a desirable control for this disease.

## DISEASE CONTROL

Even though there is considerable damage from powdery-mildew infection through the destruction of chlorophyl in bean leaves, together with

TABLE 3.—*Data showing number of bean pods examined, number and percentage of pods infected by powdery mildew where various sprays and dusts were used as controls for the years 1931 and 1932*

| Fungicide | Pods examined | Pods infected | Pods infected |
|---|---|---|---|
| | *No.* | *No.* | *Per cent* |
| Nontreated check | 2795 | 1498 | 53.6 |
| Sulphur dust 100 per cent | 877 | 0 | 0.0 |
| Sulphur-lime 75–25 | 1002 | 13 | 1.2 |
| Sulphur-lime 50–50 | 1003 | 15 | 1.4 |
| Sulphur-lime 25–75 | 1003 | 18 | 1.7 |
| Manganar rose dust 85 per cent sulphur | 363 | 8 | 2.2 |
| Copper-lime dust 25–75 | 1114 | 456 | 40.9 |
| Koppers sulphur dust 100 per cent | 402 | 0 | 0.0 |
| Lime-sulphur spray | 377 | 9 | 2.3 |
| Bordeaux mixture 4–4–50 | 346 | 59 | 17.0 |
| Flotation sulphur spray 1–160 | 319 | 5 | 1.5 |
| Sulphur-aluminium stearate dust 95 per cent sulphur | 650 | 0 | 0.0 |

some defoliation, the major economic losses are from pod spots. The principal aim, therefore, in controlling leaf, petiole, and stem infection is to protect the developing pods from infection. Since pod infection occurs considerably later in the season than does foliage infection and, since pod infection probably comes largely from the foliage, stems, and petioles, it is reasonable to expect pod-spot control to parallel very closely that of foliage infection. This is clearly seen from the data in table 3. Due to the fact that pod spot developed on the experimental plots in only 2 of the 5 years this work was done, the data are not so extensive as in the case of foliage infection. It is apparent, however, from the information collected that 100 per cent sulphur as a dust, 75–25 sulphur-lime dust, 100 per cent Koppers Sulphur dust, lime-sulphur spray or flotation sulphur spray 1–160 will give adequate control.

## RELATION OF WEATHER TO DISEASE INCIDENCE

Powdery mildew occurs every year on beans in the Southeastern United States during the fall months, but rarely does it appear on the spring crop. The reason for this, though probably a matter of speculation, is, nevertheless, of some interest and importance. The number of spores is somewhat limited during the spring months as compared with the fall months, because of the earliness of the bean crop, the harvest period ranging from late April through May. Few of the hosts of the causal organism are developed at this time, consequently there is not a general distribution of spores. During

the fall months, however, there are numerous plant species, such as those from the genera Brassica, Trifolium, Vigna, Pisum, Medicago, and others, on which the same species of mildew develop early in September, thus furnishing ample spore material for a general field infection. With this natural condition prevalent, the factors limiting bean infection in the fall would logically appear to be either humidity or air temperature or both.

TABLE 4.—*Showing dates of mildew infection on beans for the years 1931–1935, date of rainfall, and elapsed time until infection occurred*

| Rainfall | Rainfall | Mildew infection | Days between rainfall and infection | Mean temperature 10 days before infection | Mean temperature for month of May |
|---|---|---|---|---|---|
| *Date* | *Inches* | *Date* | | °*F.* | °*F.* |
| 10/ 9/31 | 0.18 | 10/13 | 4 | 73.8 | 71.4 |
| 10/ 5/32 | 0.53 | 10/ 8 | 3 | 69.6 | 72.6 |
| 10/ 9/33 | 0.13 | 10/16 | 7 | 67.8 | 77.9 |
| 10/ 1/34 | 1.77 | 10/ 4 | 3 | 76.0 | 71.0 |
| 9/19/35 | 0.20 | 9/24 | 5 | 76.0 | 73.6 |

In table 4 are shown the first date of observed mildew infection for each year from 1931 to 1935, inclusive, together with date of first rainfall and amount of rainfall prior to infection and the mean temperature for the 10-day periods immediately preceding infection. For comparison the mean monthly temperatures for May are shown for each of the above years. Here it appears that a correlation exists between humidity and disease infection during the fall bean season. Temperature conditions at all times seem to be within the range favorable for fungus development. With an abundance of spore material present on both cultivated and wild host plants, the spread to and the development on the bean plants seems dependent only upon a requisite amount of rainy weather. However, the data presented are too limited to warrant a definite conclusion on this point and are included to show only an apparent relationship.

### VARIETAL SUSCEPTIBILITY

Although all of the known commercial varieties of snap beans are susceptible to powdery mildew, there is a wide difference in susceptibility. In table 5 are listed, according to severity, 33 of the better-known varieties on which observations of natural infection were made during the period of this investigation. Here it is seen that Asgrow Valentine and Sure Crop Wax showed light infections of powdery mildew, while Giant Stringless

TABLE 5.—*Showing the relative susceptibility of various dwarf varieties of beans to infection by powdery mildew*

| Variety | Class | Kind of pod |
|---|---|---|
| **Light infection**[a] | | |
| Asgrow Valentine | Garden | Green |
| Dwarf Horticultural | " | " |
| French Horticultural | " | " |
| Great Northern | Field | " |
| Grenell Rustproof | Garden | " |
| Hodson Wax | " | Wax |
| Keeney Rustless Wax | " | " |
| Prolific Black Wax | " | " |
| Round Pod Kidney Wax | " | " |
| Stringless Green Refugee | " | Green |
| Stringless Kidney Wax | " | Wax |
| Sure Crop Wax | " | " |
| **Moderate infection** | | |
| Black Valentine | Garden | Green |
| Burpee Stringless Green Pod | " | " |
| Currie Rustproof Wax | " | Wax |
| Davis White Wax | " | " |
| Fordhook Favorite Bush | " | Green |
| Full Measure | " | " |
| Giant Stringless Green Pod | " | " |
| Improved Golden Wax | " | Wax |
| Konserva | " | Green |
| Longfellow | " | " |
| Pencil Pod Black Wax | " | Wax |
| Red Valentine | " | Green |
| Unrivalled Wax | " | Wax |
| Wardwell Kidney Wax | " | " |
| Weber Wax | " | " |
| **Severe infection** | | |
| Bountiful | Garden | Green |
| Extra Early Refugee | " | " |
| Low Champion Bush | " | " |
| Refugee 1000–1 | " | " |
| Refugee Wax | " | Wax |
| Tennessee Green Pod | " | Green |
| White-seeded Refugee | " | Wax |

[a] Infections from a trace to 25 per cent are listed as "light"; from 25 to 75 per cent as "moderate"; from 75 to 100 per cent as "severe."

Green Pod, Black Valentine, and Konserva were moderately infected. Bountiful was the only important southern commercial variety that was severely infected. It should be noted, however, that either a light or a moderate infection may be of considerable economic importance, since infected bean pods were found on plants that showed only a limited amount of foliage infection, as well as on those severely infected. While this grouping of varieties is arbitrary and subject to some change from year to year, it does suggest a degree of varietal resistance that may be of importance in a program of plant breeding.

## DISCUSSION OF RESULTS

Powdery mildew attacks the fall crop of beans throughout the Southeast. It seldom is observed on the spring crop, although both rainfall and mean temperatures for May and October (Charleston, S. C., readings) are so nearly the same as to indicate that factors other than weather may cause the difference in mildew infection. The wide distribution during the fall of natural host plants of *Erysiphe polygoni*, which produce an abundance of spores during the later stages of bean growth, seems to account for this seasonal occurrence. That only a limited amount of rainfall is necessary for infection is indicated from the weather data collected over the 5-year period covered by this work. It is not unlikely that the length of the period of high humidity during a rain or following it might be in part at least the factor controlling infection.

Since powdery mildew may be expected to appear in bean fields throughout the Southeast each fall, and since infection consistently follows rains during late September and early October, the time for inaugurating control measures can be fairly well ascertained. The data collected on control of powdery mildew indicate that either sulphur sprays or sulphur dusts may be used, but that the sulphur-lime dust (75 per cent sulphur, 25 per cent lime) is more effective, easier to handle and more economical than the sprays. Good control may be had by making the first application when the first signs of the disease appear on the foliage, and repeating at 10-day to 2-week intervals thereafter. Usually, from two to three applications should be sufficient.

## SUMMARY

Powdery mildew is a widely distributed disease of snap beans, and, in recent years, has become of considerable economic importance throughout the Southeast.

The disease is rarely found on the spring crop, but appears each year on the fall crop.

Fungus development is closely associated with moisture conditions, usually following upon light rains in late September and early October.

Good control of the disease may be obtained by application of either sulphur dusts or sulphur sprays. The sulphur-lime 75–25 dust proved to be the most economical and efficient material tested.

# A VIRUS DISEASE OF PRUNE

H. Earl Thomas and E. M. Hildebrand

(Accepted for publication March 23, 1936)

During the late summer of 1930, attention was called to a disease of prunes in Niagara County, New York, which was accompanied by certain symptoms characteristic of virus diseases. Thus far these symptoms have been seen occurring naturally only in Niagara County and on the variety Fellenberg. During the season of 1931, the disease was found in 4 orchards affecting from 1 or 2 trees to 18 per cent in one small block of 58 trees. In 1934 a single diseased tree was found in another orchard. On one farm, trees of cherry, peach, and plum were growing within 30 feet of a severely affected prune tree, but no symptoms suggestive of a virus disease were seen on these. According to accounts of growers who report having had affected trees under observation for as long as 19 years, the disease seems to spread very slowly in the orchards of this district. Notwithstanding this fact and the small total number of trees now known to be involved, the disease finally renders the tree virtually worthless and constitutes a menace to prune culture in areas that may afford a more effective agent of dissemination.

*Symptoms.* The affected leaves are reduced in size and distinctly narrowed in proportion to length. Serration and pubescence are suppressed, and there is considerable rugosity and mottling (Fig. 1, B). The rugosity is more marked near the midrib than toward the margin of the leaf. The leaf blade is somewhat thickened. Leaf margins are frequently so irregular in outline as to resemble, under casual observations, the effect of chewing insects. The surface of the severely affected leaf presents a somewhat glazed aspect. All the leaves on a given shoot are affected, those at the base somewhat more so than those at the tip. The shoot may grow for several inches in a season or only a fraction of an inch. Internodes are shortened in varying degrees. An unusual feature of this disease is the development of a shoot that remains perfectly normal in appearance throughout the season, in the midst of buds that produce only severely affected foliage. Larger branches may be apparently normal and fruitful on trees that, otherwise, appear to be entirely involved by the disease; but this may be due to the fact that the virus has not yet invaded the entire tree. Diseased trees may blossom profusely, but only an occasional fruit matures on the parts that exhibit foliage symptoms. Pistils are aborted in many of the blossoms and petals are somewhat narrowed and irregular in shape (Fig. 1, B). The time of blossoming and of maturity of fruit seems not to be influenced by the disease, nor does the quality of the few fruits that reach maturity.

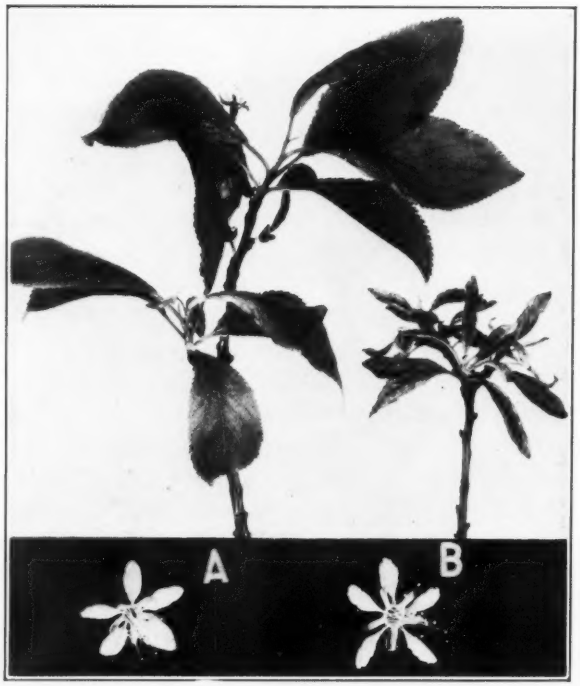


FIG. 1. A. Healthy prune leaves and blossom. B. Naturally infected prune branch and blossom from a diseased branch.

*Infection experiments.* On January 21, 1931, 10 cions from affected Fellenberg trees were grafted on 3 two-year-old German prune trees and these were planted at once in the greenhouse. Two trees of the same lot were planted as controls. Only 2 of the cions lived long enough to develop foliage, but symptoms appeared at or near 6 of the 10 points of inoculation. These were first seen nearly 60 days after inoculation, and at that time appeared only as a vague mottling in the leaf blade. Later, on some leaves, there appeared a very definite mottling and, on others, a distinct line pattern. Except on 1 of the 2 surviving cions, the typical narrowed and dwarfed leaves seen in the orchard were not produced in the first growth cycle. Shoots from buds more remote from the points of inoculation grew until August, 1931, some of them reaching 3 feet or more in length. Several of these appeared to be normal in all respects, while the leaves of others became distinctly rugose and mottled.

On May 21, 1931, buds from an infected shoot were set into 2 larger branches of a 15-year-old Fellenberg tree in the orchard. No infection was apparent on this tree on September 18, 1931. The abundance of red mites on the foliage at this time, however, would have obscured any but the more obvious symptoms. This tree was removed in 1932. In September, 1931,

John Goodrich inoculated a 22-year-old Fellenberg tree on the same farm by budding on a single terminal shoot. Symptoms were apparent on the inoculated branch in 1933, and, in 1934, they had spread only to about 1 foot below the point of inoculation.

Another experiment was begun on May 22, 1931. Buds from an affected prune tree were set into the following nursery trees: 2 cherry (Montmorency), 1 peach, 2 plum (Lombard), 2 prune (known locally as Smith's prune). Two buds were set into each tree in vertical line and covered with paraffin. The trees were then planted on the University Farm at Ithaca. One tree each of cherry, plum, and prune were planted as checks. None of the buds survived long enough to produce new leaves. The trees set in poor orchard soil made a short growth cycle followed by a brief second cycle during the latter part of the summer. No certain symptoms were seen on any of these during 1931. However, in July, 1932, definite symptoms were found on the inoculated prune and plum strees, resembling those of naturally infected orchard trees. The symptoms had become severe on the prune trees in October, 1934, and were marked on the plum trees. In 1935 the plum trees bore approximately a normal crop of fruit, while the prune trees bore less than one-fourth of a crop. The check trees and the inoculated cherry and peach trees remained healthy in appearance.

The preceding experiment was repeated in 1932 with essentially the same results. Inoculations were made in early August, 1932, and symptoms began to appear in early July, 1933. Again, prune and plum became infected, while cherry and peach did not. Further attempts are being made to transmit the disease to peach.

In a single experiment, 8 colonies of the green plum aphid (*Myzus Mahaleb*) were transferred from infected shoots and confined in cellophane bags on healthy prune and plum shoots. In another test, 4 colonies of *Aphi setariae* were similarly transferred from diseased to healthy shoots. In these trials, started in May, 1933, no infection resulted up to October, 1934, when the last observation was made.

The virus seems to spread rather slowly through the inoculated tree, as is the case with many virus diseases of woody plants. In this instance, judging by the time and place of the appearance of symptoms, the virus seems to move rather rapidly upward in line with the point of inoculation, distinctly less rapidly downward, and very slowly laterally.

### RELATION TO DISEASES OF PRUNE AND PLUM IN OTHER AREAS

The locality (western New York) in which this disease is found is well beyond the present known range of plum rosette[1] and plum mosaic.[2] Moreover,

[1] McClintock, J. A. Peach rosette, an infectious mosaic. Jour. Agr. Res. [U. S.] **24**: 307–315. 1923.

[2] Valleau, W. D. A virus disease of plum and peach. Kentucky Agr. Expt. Sta. Bull. 327. 89–103. 1932.

the prune disease seems to differ from these in symptoms, is less severe on plum, and fails to affect the peach. One of the writers has seen leaf symptoms on Santa Rosa plum in central California very similar to those shown for plum mosaic in Kentucky (Page 95, fig. 23).[2] In this (California) instance there was no noticeable reduction in size of leaves or in vigor of the trees 2 years after the symptoms were first seen. Symptoms resembling those of the prune disease have been described for prune in Holland,[3] but are said to appear also on cherry and peach. No inoculation experiments are reported. No relation is apparent between the prune disease in New York and the group of diseases discussed by Atanasoff under the title "Mosaic disease of drupaceous fruit trees."[4]

Given little (or nothing) beyond symptoms as a basis for comparison, and these in some cases not fully described, it cannot now be determined with finality whether the disease under discussion is distinct from all those previously described. It seems unlikely, however, that this disease will prove to be identical with any thus far studied in plum or prune in North America.[5]

Under the observed conditions of limited incidence and slow rate of spread the disease should be amenable to control by eradication.

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[3] Rietsema, I. J. Weinig bekende ziekten in kers, pruim, en perzik. Tijdschr. Plantenziekten. 36: 261–269. 1930.

[4] Atanasoff, D. Mosaic disease of drupaceous fruit trees. God. Sofiisk. Univ. Agron. Lesov. Fakult. (Ann. Univ. Sofia, Facult. Agron. et Sylvic.) Livre I. Agron. 13 (1934/1935): 9–42. 1935.

[5] There are now probably 4 virus diseases of plum and prune in the United States in addition to the peach diseases of "Yellows" and "Rosette," which also affect plums. In an unpublished paper presented by Donald Cation at the 1935–1936 A. A. A. S. meetings in St. Louis, entitled "A rosetted-mosaic of peach and plum trees," evidence on a new virus disease in Michigan was presented. This disease appears to be distinct from "peach mosaic" found in Colorado and "peach rosette" in the south. The junior writer was shown peach trees severely affected with rosetted-mosaic by Mr. Cation in 1933. According to Mr. Cation it usually is masked in the European plum varieties, but Burbank and Damson trees are very susceptible, showing a striking loss of vigor. Working with material similar to that of Valleau, Cation also has obtained evidence that Valleau probably was dealing with two plum viruses, one of which transmits to peach trees and causes a striking leaf pattern, while the other does not.

# ENDOXEROSIS OF LEMON FRUITS AS AFFECTED BY THE APPLICATION OF DIFFERENT AMOUNTS OF IRRIGATION WATER[1]

E. T. Bartholomew[2]

(Accepted for publication March 30, 1936)

Endoxerosis[3] is an internal, nonpathogenic disorder, which appears in many lemon fruits as they approach or reach maturity. The first symptoms appear in or near the terminals of the vascular bundles in the peel at the stylar end of the fruit. As the disorder spreads, it may be found in isolated areas in any portion of the albedo of the peel. Concurrently, it begins to appear in the pulp at the stylar end and gradually spreads toward the stem end of the fruit. Gum is usually in evidence and the affected tissues show a pinkish yellow to a rust brown discoloration. Many of the affected cells, especially in the pulp, lose their water and disintegrate or collapse, leaving air spaces; hence the term endoxerosis (internal drying). By the time one-third to one-half of the pulp tissues are affected, the fruit usually falls from the tree.

Endoxerosis of lemon fruits has been reported from Sicily by Savastano (9) and from Australia by Jarvis (8). However, the symptoms for endoxerosis in these countries do not entirely agree with those for California.

For a more complete discussion of the distribution, characteristics, and contributing causes of endoxerosis, see Bartholomew, Barrett, and Fawcett (1), Bartholomew (2, 3, 5, 6), Bartholomew and Robbins (4), and Fawcett and Lee (7).

Previous observations and experiments had indicated that endoxerosis of lemon fruits might be largely influenced by the excessive withdrawal of water from the fruits by the leaves. Such a withdrawal would occur during periods of comparatively high temperature and low relative humidity in late spring and during the summer months. The experiment described in this paper was made in order either to disprove or substantiate this possibility.

## MATERIALS AND METHODS

Twelve Eureka lemon trees, *Citrus limonia* Osbeck, budded on sour-orange stock, *C. aurantium* Linn., were used in this experiment. These

[1] Paper No. 342, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] The author wishes to acknowledge his indebtedness to those who assisted in the experimental work, and especially to Mr. E. C. Raby, who made most of the soil-moisture determinations and gave other valuable assistance.

[3] The term "endoxerosis" replaces "internal decline," the term used in earlier publications.

trees were grown in lysimeter tanks buried in the ground. The tanks were 10 feet in diameter, 4 feet deep at the edge, and 4.5 feet deep in the center. A detailed account of the special attention given to tree propagation, soil selection, soil-moisture determinations, and water applications will not be given here. These details are being published elsewhere.

The trees were planted in May, 1927, and from then to the end of 1934 all trees received the same amounts of pruning, soil fertilizers, and cultivation. In May, 1931, tests showed the apparent specific gravity of the soil in all tanks to be 1.5, the moisture equivalent approximately 11 per cent, and the wilting point 3.5 to 4 per cent.

The trees were divided into 3 groups of 4 trees each. The grouping was based upon the total production of endoxerotic fruits by each individual tree between January, 1929, when fruit production began, and May, 1931. During this period all trees received like amounts of water.

A different amount of water was applied to each group of trees between June, 1931, and December, 1934. Water was applied when the total mass of soil in each tank had reached an average moisture content of 6 to 8 per cent in group 1, 5 to 6 per cent in group 2, and 4 to 5 per cent in group 3. Sufficient water was applied to the soil in each tank at each irrigation to bring its entire mass up to an average moisture content of 11 per cent. The water was measured in and applied from a calibrated reservoir adjacent to each tank. The total number of gallons of irrigation water applied was 47,677 to the trees in group 1; 44,920 in group 2; and 38,545 in group 3.

Rainfall, measured in a rain-gauge that stood near the experimental group of trees, was taken into consideration in applying irrigation water.

Most of the fruit was picked according to regular ring-size method, which means that each fruit had attained a transverse diameter of at least 2.25 inches before it was picked. However, endoxerotic, windfall, tree-ripe, and sunburned fruits that did not attain full ring size were included in the tree yield. Total individual tree yields were also weighed at each picking.

## RESULTS

The principal results obtained in this experiment are shown in table 1. The data in the table show that before differential water treatments were begun, the fruit production of groups 1 and 3 was practically the same, while that of group 2 was somewhat less. While the total production of good fruit over the entire period from 1929 to 1934 was greatest for group 1, the increase in yield during the second period (period of differential water treatment) was greatest for group 2. The increases in yield of good fruit for the second period were 575, 676, and 190 pounds, respectively, for groups 1, 2, and 3.

TABLE 1.—*Proportions of good and endoxerotic fruit produced by experimental tree groups, 1929–1934, inclusive*[a]

| Group | 1929–31 | | | 1932–34 | | | | |
|---|---|---|---|---|---|---|---|---|
| | Condition of fruit | | | Soil moisture content[b] | Irrigation water applied | Condition of fruit | | |
| | Good | Endoxerotic | | | | Good | Endoxerotic | |
| | *pounds* | *pounds* | *Per cent* | *per cent* | *gallons* | *pounds* | *pounds* | *Per cent* |
| 1 | 1,389 | 137 | 9 | 6–8 | 47,677 | 1,984 | 245 | 11 |
| 2 | 1,219 | 151 | 11 | 5–6 | 44,920 | 1,895 | 211 | 10 |
| 3 | 1,414 | 106 | 7 | 4–5 | 38,545 | 1,604 | 328 | 17 |

[a] Differential water treatments were begun in June, 1931.

[b] The figures in this column indicate the amount of moisture in the soil each time that irrigation water was applied. For the period 1929–31, all trees received like amounts of water.

During the period over which all groups received equal amounts of water, group 2 produced the greatest percentage of endoxerotic fruits and group 3 the least. During the second period (1932–34), group 2 produced about the same percentage of affected fruits as during the first period, while there was a slight increase in group 1, and a comparatively marked increase in group 3.

A single picking on August 6, 1934, showed results similar to those for the 1932–34 period in table 1. In this picking, 561 of the fruits from the trees in group 1, 459 from group 2, and 1,915 from group 3 were endoxerotic. These figures represent 40, 37, and 62 per cent, respectively, of the fruit picked from the 3 groups on that date.

It is of interest to note also that the sizes of the endoxerotic fruits for the period 1932–34 were smaller for group 3 than for either of the other 2 groups. For example, during the period 1931 to 1934 it took an average of 7.3 endoxerotic fruits from the trees in group 1, 6.6 from group 2, and 8.5 from group 3 to weigh 1 pound.

## DISCUSSION

That there is an element of danger in attempting to interpret results obtained from as small a population as only 12 trees, 4 in each group, is fully realized. However, it should be remembered that the trees were carefully propagated from known sources and were grown under controlled conditions; the group differences in these tests, though not large, are positive; and the results confirm those of previous tests and observations. Such factors make the conclusions much more reliable than if the trees had been grown and cared for under the usual field conditions.

The difference shown in table 1 between 11 per cent endoxerotic fruits for group 1 and 10 per cent for group 2 for the second period is negligible, but it becomes more significant when compared with 9 per cent and 11 per cent, respectively, for the first period of the treatment. It becomes even more significant when compared with the results obtained in the specially mentioned pick of August 6.

As shown in table 1, the production of good fruit in group 1 went from second place in the first period to first place in second period, while group 2 went from third place to second place. Based on production during the first period, however, not only the yield of good fruit, but also the total yield of group 2 exceeded that of group 1 during the second period. These results indicate that the greatest yield of good fruit may be obtained when a moderate amount of irrigation water has been applied and that this maximum yield may be obtained without the appearance of any more, and possibly less, endoxerosis.

Many fruits attain full size before showing an endoxerotic condition. However, that many of the fruits may be affected before they become mature is shown by the fact that during the period 1931 to 1934 an average of 7.3, 6.6, and 8.5 endoxerotic fruits from groups 1, 2, and 3, respectively, were required to make 1 pound in weight. The fact that for the pick of August 6, 1934, it took 9.4 endoxerotic fruits from group 3 to weigh a pound indicates in a marked way that a deficiency of soil water causes smaller fruits to be affected. These fruits may have been small because they were young or because they were slow-growing and stunted. It takes an average of 4 fruits of the usual picking size to weigh 1 pound.

The results reported in this paper indicate that the development of endoxerotic conditions in lemon fruits is influenced by the withdrawal of water from the fruits by the leaves during periods of excessively warm weather. However, the withdrawal of the water from the fruit by the leaves is not the only factor concerned. A comparatively high temperature, aside from its effect on the rate of transpiration, is necessary. Furthermore, the presence of substances that may be converted into gums, hexosans (4), appears to be an important factor. It would seem that a little more of these substances was produced in the fruit of group 1 and much more in the fruit of group 3 than in that of group 2. This agrees with the previous observations that young, thrifty trees with a comparatively large unit of transpiring surface per fruit produce more endoxerosis than those with a moderate amount of foliage; but the greatest amount of endoxerosis is found on trees that over a period of years have not had enough water.

The experimental results indicate that the withholding of water from lemon trees until the moisture content of the soil had almost reached its

wilting point produced cumulative effects in the trees and fruit. This is shown by the fact that, although the trees in group 3 had received an irrigation that brought the soil mass in each tank up to its full moisture equivalent at the beginning of the hot period of 7 days, the pick made from this group, 4 days after the close of the hot period, showed 62 per cent of the fruit to be endoxerotic. Incidentally, the amount of endoxerosis has been observed to increase from summer to summer after a series of low winter rainfalls.

## SUMMARY

Three groups of trees were grown in tanks in soil having a moisture equivalent of 11 per cent and a wilting point of 3.5 to 4 per cent. During the first period of the experiment the trees received like amounts of water, while during the second period they were not irrigated until the soil mass in the tanks had reached a moisture content of 6 to 8 per cent in group 1, 5 to 6 per cent in group 2, and 4 to 5 per cent in group 3.

The trees in group 3, which received the fewest irrigations, and the least amount of water, produced the greatest quantity of endoxerotic fruit and the greatest number of fruits that became endoxerotic before they reached picking size.

During the period of differential water treatment the trees receiving the medium amount of water produced the greatest amount of good and the least amount of endoxerotic fruit.

The results of these tests substantiate previously obtained evidence to the effect that the withdrawal of water from the fruit during periods of excessively high temperature and low relative humidity is an important factor in the production of endoxerosis.

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## LITERATURE CITED

1. BARTHOLOMEW, E. T., J. T. BARRETT, and H. S. FAWCETT. Internal decline of lemons. I. Distribution and characteristics. Amer. Jour. Bot. **10**: 67–70. 1923.
2. BARTHOLOMEW, E. T. Internal decline of lemons. II. Growth rate, water content, and acidity of lemons at different stages of maturity. Amer. Jour. Bot. **10**: 117–126. 1923.
3. ————————. Internal decline of lemons. III. Water deficit in lemon fruits caused by excessive leaf evaporation. Amer. Jour. Bot. **13**: 102–117. 1926.
4. ————————, and W. J. ROBBINS. Internal decline (endoxerosis) of lemons. IV. The carbohydrates in the peel of healthy and endoxerotic fruits. Amer. Jour. Bot. **13**: 342–354. 1926.

5. BARTHOLOMEW, E. T. Internal decline (endoxerosis) of lemons. V. Concerning the comparative rates of water conduction in twigs and fruits. Amer. Jour. Bot. **15**: 497–508. 1928.
6. ————————. Internal decline (endoxerosis) of lemons. VI. Gum formation in the lemon fruit and its twig. Amer. Jour. Bot. **15**: 548–563. 1928.
7. FAWCETT, H. S., and H. A. LEE. Citrus diseases and their control. 582 pp. McGraw-Hill Book Co., Inc., New York. 1926.
8. JARVIS, E. A gumming disease affecting lemon fruits. Queensland Agr. Jour. (n.s.) **1**: 345–348. 1914.
9. SAVASTANO, G. L'endoxerosi del limone in Sicilia. Boll. R. Staz. Patol. Veg. [Roma] (n.s.) **12**: 169–186. 1932.

## PHYTOPATHOLOGICAL NOTE

*Spore Formation and Discharge in Fomes fomentarius.*—In March, 1930, at the Laboratory of Phytopathology of the Central Forest Experiment Station (Moscow, U. S. S. R.) there was obtained a green block of lime wood seriously injured by the fungus *Fomes fomentarius.* On the surface of the block there were two well-developed fruit bodies of the fungus. The block of wood was so placed on a stand in the corner of the room that one of the fruiting bodies, situated at the top, faced the window, while the other, situated considerably below on the other side, was 12 cm. from the pipes of the central heating system.

Presently there appeared on the lower surface of the fruiting body of the fungus a thin white layer, at first on the more heated side and afterwards over the whole surface.

Five or six days after the first signs of this phenomenon, the entire fruiting body of this fungus was covered with a thick layer of white spores. They were oblong, measuring $10–18 \times 4–6\ \mu$. The spores, on being discharged from the hymenium, apparently were drawn upwards by the movement of the heated air and precipitated on the colder surface of the fruiting body.

Spore discharge of *Fomes fomentarius* was observed during approximately one month. The precise moment of their appearance was not determined. On March 25 they already covered the whole surface of the fruiting body and by April 15 their development was discontinued. It is possible that in nature spore formation proceeds more slowly than under laboratory conditions, where the gradual drying of the wood doubtless inhibited their further development.

The spores were collected abundantly every day and weighed. From March 25 until April 13 there were collected a total of 1,115g. This figure does not represent the total amount, as at the outset the spores were not collected and naturally some of them may have been carried away by the movement of the air. Others may have been lost while collecting.

In order to determine the approximate number of spores discharged during the time of their collection (20 days), they were computed as follows: The average size of a spore is $14 \times 5\ \mu$; assuming the shape of a spore as an ellipsoid, it is possible with the help of a formula of rotation of an ellipsoid around its large axis to find the volume of one spore: $V = 4/3\pi b^2 \cdot a$ (a–a large, b–a small semiaxis). Its volume equals 163 cb. $\mu$. Assuming then that the specific gravity of one spore is approximately that of water and may be taken as 0.9, we find the weight of one spore. It is equivalent

to 0.000,000,000,146 gr. Dividing the $\frac{1{,}115}{0.000{,}000{,}000{,}146}$ we get the number of spores discharged within 20 days, that is from March 25 until April 13. This number is equivalent to 7,563,493,150. During a 24-hour period the single fruiting body of *Fomes fomentarius* under observation discharged 378,424,655 spores.

The above mentioned computations roughly approximate the truth, but they, nevertheless, indicate the probable number of spores discharged by a single fruit body of *Fomes fomentarius*.

The pure culture consisting of collected spores of *Fomes fomentarius* had the usual macroscopic appearance, *i.e.*, a continuous felt-like mycellium, smooth, at first yellowish, then brown. Growth of culture is moderately rapid. Among the hyphae of the aerial mycelium there are mostly fiber-like 1–4 μ in diam., thick-walled, poorly ramified, continuous hyphae, often a golden chestnut color.

The hyphae of the submerged mycelium have thin walls and are 2–7 μ in diam. with strands, continuous, ramified.

The spores possessed a high capacity for germination. When sown in the Petri dish on agar-malt medium, they formed on the next day an abundant growth of mycelium. They germinated rather well on the water-soaked block of wood. The germinating power of spores is of a rather long duration. The spores kept in the laboratory in cotton-plugged test tubes germinated after 5 months as well as newly collected spores. Tested in March, 1931, one year after collection, they showed 25 per cent of germinating power.—HELEN MEYER, Centr. Forest Experiment Station, Moscow, U. S. S. R.

## BOOK REVIEW

Raillo, A. I. Diagnostic estimation of morphological and cultural characters of species in the Genus Fusarium. [In Russian with English title.] 100 pp. illus. Inst. Zashch Rast. Trudy Zashch. Rast (Leningrad Acad. Agr. Sci. U.S.S.R., Inst. Plant Prot. Bull. plant protect.) series 2 Phytopath., No. 7. Published by the Lenin Acad. of Agr. Sci., Leningrad, 1935. Price 3 rbls.

This interesting publication by Miss Raillo presents a decisive departure from the customary method of approach to the classification of the genus Fusarium. Heretofore, the author points out, no complete and proper evaluation of the various taxonomic characters, typifying different subdivisions of the genus, has been made and, consequently, no clear concept of the structure of species and its dynamics could have been formed. Certain features have been indiscriminately used for the differentiation of higher units in one case and lower in another. This confusion is regarded as the chief reason for the great difficulties that are encountered by practically everyone in attempting to identify or classify Fusarium cultures. These difficulties are further augmented by the fact that, as a rule, different workers have used different technique, and thus no comparison of results is possible.

Raillo's objects were: (1), to thoroughly standardize methods of procedure; (2) to estimate the diagnostic value of each character, and (3) to draw up a scheme of the structure of species and of its dynamics.

The standardized procedure employed by Raillo was as follows: 50 *single-spore* cultures were used for every taxonomic unit studied and on each of the 5 kinds of *media* employed (potato agar and acid potato agar for fruiting, rice and potato plugs for pigment and, in some cases, to check up on certain behavior, also the synthetic medium of Leonian). All cultures were held under a diffused light at the temperature between 22° and 25° C. *Measurements* of the conidia were made on the 15th day, provided the typical fruitification was present; otherwise, on the 30th or 45th day, etc., so that no spores were used older than 15 days; 100 conidia with the prevailing number of septae were measured in each culture and all measurements were treated statistically. *Drawings* were made at the time of *measurement*, with a uniform magnification of x 1000. The description of *pigment* development was made on the 15th and the 30th day, using Ridgway's system of color nomenclature and painting, on the 30th day.

No question is raised as to the validity and clearness of Wollenweber's characterization of the sections in the genus Fusarium. Moreover, the precise determination of the section to which a given Fusarium culture belongs, is considered to be the first and indispensable step in the identifica-

tion of this culture. The critical revision and revaluation concern those taxonomic features that characterize the species and its subdivisions. In particular:

*Length and width of macroconidia.* These characters occupy more or less dominant position in existing systems, but Raillo gives them only a subordinate value. A study of the amplitude of variations in the length of the conidia and of the ratio between the difference of means of two extreme rows and their mean error, convinces her that the length cannot serve as a diagnostic character for higher taxonomic units, unless the amplitude of variations is given. The length measurements of conidia of one isolate may characterize only this isolate, or at the most, the race. The width measurements, likewise, show unequal value of different conidia, and, therefore, are not regarded as distinguishing for higher taxonomic units unless the amplitude of fluctuations is given.

*Septa.* Wollenweber and other workers used the number of cross walls as a fundamental character in distinguishing species and varieties, but Raillo finds that too great variations exist, even for different isolates of the same single-spore cultures. She studied the distribution of the number of septa among 100 observations for each culture and concluded that only the prevailing number may be used for characterization of a taxonomic unit.

The *shape of the conidia* is a complex character and is determined by the three component parts: (a) form of the apical cell, (b) length of the apical cell, and (c) incurvation of the conidia. These three features are evaluated separately.

(a) *Form of the apical cell* has heretofore received very insufficient attention, but Raillo finds that this is the only constant character for all single-spore cultures of a given species and, therefore, diagnostic for the species within each section. The terms for the designation of shape variations of the apical cell are adopted from the Wollenweber's system, and are used to denote the manner and the degree of tapering. Accordingly, the following 7 types are recognized by the author: (1) slightly narrowed and rounded, as in *F. solani* (Mart.) App. et Wr. (section Martiella); (2) suddenly narrowed as in *F. culmorum* (W. G. Sm.) Sacc. var. *lethaeum* Sherb. (section Discolor); (3) gradually and evenly narrowed, as in *F. equiseti* (Cda.) Sacc. (section Gibbosum); (4) same as (3) but truncated, as in *F. lateritium* Nees var. *majus* Wr. (section Lateritium); (5) sharply and considerably narrowed, as in *F. scirpi* Lamb. et Fautr. (section Gibbosum); (6) filiform, as in *F. scirpi* Lamb. et Fautr. var. *filiferum* (Preuss.) Wr., and (7) same as (6) but crooked, as in *F. scirpi* Lamb. et Fautr. var. *caudatum* Wr.

(b) *Length of the apical cell* has diagnostic value only in those cases when its elongation changes the morphology of the conidia, which may be

seen in the case of gradually and considerably narrowed or filiform cells, as in *F. herbarum* and *F. avenaceum* (section Roseum). No generalization can be made on this basis, however, in regard to species in other sections without a further detailed study.

(c) *Curvature of the conidia* has been regarded by Wollenweber as a diagnostic character for varieties and forms. Raillo finds that it should be used as a distinguishing character for subspecies, since its dynamics indicate the variation within the species, but not within lower divisions. There are 5 known types of curvature for the designation of which Raillo uses terms introduced by Wollenweber, namely: (1) nearly straight, as in *F. semitectum* Berk. et Rav. (section Arthrosporiella); (2) eel-shaped, as in *F. diversisporum* (section Arthrosporiella); (3) elliptical, as in spp. of sections Elegans, Sporotrichiella, Lateritium, Liseola and others; (4) parabolic, as in *F. equiseti* (Cda.) Sacc. f. I Wr., and (5) hyperbolic, as in *F. scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr.

The author's general conclusion as regards cultural characters is that they are of taxonomic value, but for lower units only. Namely:

(a) *Pigment* on rice can characterize forms. On potato plugs and on the Leonian agar sharp differences occurred even among the single-spore isolates from one single-spore culture, showing that on these media pigment can characterize races only.

(b) *Sclerotia,* as regards their color and size, presence or absence, were found by Raillo to vary greatly even among different isolates and, consequently, characteristic of races only.

(c) The type of *fruiting* (sporodochia, pseudopionnotes and pionnotes) likewise is regarded as characteristic of races only.

The foregoing analysis tends to show that the Fusarium species is a complex system characterized by series of parallel and non-parallel features. Only one of them is found by the author to be of diagnostic value for the species, *i.e.,* the form of the apical cell, as a non-parallel character for other species in a given section. It cannot be used as a sectional character, since it is not identical for all species in the majority of sections. The various elements of morphology of the conidia may become a basis for characterization of higher taxonomic units only in their dynamics and the systematic value of complex characters will vary in accordance with variations within the species. The author illustrates this idea by schematic structures of species having different variability.

An English summary is appended to each of the two parts of the book, but these summaries are too brief to be of practical guidance to those who may wish to test out the Raillo's methods. Hence the reviewer felt justified in presenting to the readers of Phytopathology this rather extensive abstract until a complete translation becomes available.

It is understood that the author is now engaged in the preparation of a monograph of the genus, which will embody the principles advanced in this preliminary paper. It is then, as well as through the tests that other workers may make in connection with their own Fusarium problems, that these principals will find their crucial test. At this moment no final judgment can be made as to the validity of the proposed method. However, certain conclusions advanced by Raillo are obviously sound and correct, particularly as regards the necessity for any Fusarium specialist, first of all, to estimate definitely the diagnostic value of various taxonomic characters and, subsequently, to build up a clear concept of the structure of species and the genus. It is also very evident that the deductions made from the variability data concerning the different morphological and cultural characters, are logical and point to the most confusing irregularity in their present usage. Whether the form of the apical cell is a constant character for the species in a given section can be determined only by a thorough analysis of all the known forms, but Raillo's affirmative answer may be accepted as correct as long as it does not contradict the facts. However, the terms defining the several varied forms of the apical cell in some cases leave greater precision and indisputable clarity to be desired. Thus, it would seem that such differences as exist between the types represented by *F. solani* on the one hand and *F. culmorum* var. *lethaeum* on the other, or between the apices of *F. equiseti* and *F. lateritium* var. *majus* are not clearly defined. It is possible that coinage of new terms will be necessary.

The book contains a considerable number of very excellent drawings of the types of macroconidia. It is quite regrettable that there are no colored plates illustrating the variability in pigment described by the author in the text. It is hoped, however, that they may be included in the forthcoming monograph.—MICHAEL SHAPOVALOV, U. S. Dept. of Agriculture, San Jose, California.

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